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In Memoriam

HERBERT OSBORN

The death of Professor Herbert Osborn on September 20, 1954, in his ninety-ninth year, marked the passing of the last of the great pioneers in entomology. The respect in which he was held by his fellow workers was well stated in 1939 by Doctor L. O. Howard, for many years at the head of the entomological work of the U. S. Department of Agri-

culture;—"He is the dean of the entomologists of North America, and this includes all kinds of entomologists."

He was born at Lafayette, Wisconsin, March 19, 1856, grew to manhood at Fairfax, Iowa, and attended the State College at Ames where he received the Bachelor of Science degree in 1879 and the Master's degree in 1880. He was appointed to an assistantship in zoology and entomology in 1879, advanced rapidly to a professorship in 1885, and retained that position at Ames until 1898. The nineteen years at Iowa State College were broken only by two years of further graduate study, 1881–2 at the Museum of Comparative Zoology at Harvard, and 1884–5 at the Zoological Station at Naples, Italy.

By the time, 1898, when at the age of forty-two, he was called to The Ohio State University as Head of the Department of Zoology and Entomology, he was already well known for his numerous and important publications, was State Entomologist of Iowa, a special agent for the U. S. Department of Agriculture, and President of the American Association of Economic Entomologists for 1898.

His reputation as an economic entomologist attracted numerous graduate students from various parts of the world, as well as from many states of the Union, men who have since become noted for their research and administrative ability. During these years of active teaching he was busy with many other matters; Director of the Lake Laboratory 1898–1918, Associate Entomologist at the Ohio Experiment Station, President of the Ohio Academy of Science in 1905, the American Microscopical Society in 1909–11, the Entomological Society of America 1911, the Society for the Promotion of Agricultural Science in 1916, and Managing Editor of the Annals of the Entomological Society of America from its origin in 1908 until 1929 when he withdrew in favor of Doctor C. H. Kennedy, and Director of the Ohio Biological Survey 1912–1954.

In 1916 he was relieved of administrative duties and elevated to a research professorship to provide more time for advising and directing the research programs of the ever increasing number of students in entomology. During the following years he was also a consultant at several of the state experiment stations, and of the Tropical Plant Research Foundation. This latter connection involved trips to Mexico, several of the Central American countries and Panama. These duties continued until at the age of 77 he retired as Emeritus Professor, but still his office door was always open to students who sought his advice, and his interest in promising students never ceased.

His publications, large and small, total the astonishing number of 515 items. Two of those published in earlier years as bulletins of the U. S. Department of Agriculture, "The Hessian Fly" and "Insects Affecting Domestic Animals" may be considered classics for that period. His first book "Economic Zoology" (The Macmillan Co., 1908) was re-issued several times; "Agricultural Entomology" (Lea and Febiger, 1916) was widely used, and "Meadow and Pasture Insects" (The Educator's Press, 1939), the results of a life-time of work, was an exceptionally fine and scholarly contribution to the literature of entomology. His research papers dealt chiefly with the taxonomy and life histories of the Hemiptera, especially the "leaf-hoppers," revising many of the genera and describing a great number of new species.

In his later years, no longer able to use the microscope, he devoted his time to the production of three books; "Fragments of Entomological History," Part I in 1937, and Part II in 1946, and his final work, "A Brief History of Entomology" in 1952 at the age of 96 (all by the Spahr & Glenn Co.).

Naturally he received many awards and honors during his long active career. In 1916 his alma mater granted him the degree of Doctor of Science, and in 1930 the University of Pittsburgh and again in 1936 The Ohio State University honored him with the Doctor of Laws. He was a member of numerous state and national scientific societies and a life member of the Societé Entomologique de France.

As a man he was modest, gentle, companionable, with a fine appreciation of humor, and only those who worked with him in the field were able to realize the breadth of his knowledge and interest in all phases of natural history. Unfortunately, due to the increasing necessity for narrower specialization as science progresses, we may not have the opportunity to "look upon his like again."—Raymond C. Osburn, November 8, 1954.

CHANGES IN THE THIAMINE CONTENT OF ADULT WORKER HONEYBEES ON PROTEIN-FREE DIET[1, 2]

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No extensive study has been made on the changes in the vitamin content of insects in their successive periods of development. Haydak and Vivino (1943) studied the thiamine, riboflavin, pyridoxine, niacin, pantothenic acid and ascorbic acid content of young and old stadia of honeybees under normal conditions of growth and development and found that the vitamin content, in general, decreased somewhat with the age of the insect.

In the life of a normal colony of honeybees there may be a period when the inmates of the hive have to live on a pure carbohydrate diet for a considerable period of time. This study is an attempt to determine what changes, if any, occur in the thiamine content of the bodies of adult worker honeybees at such times.

Method

During a three-day period young emerged workers, not more than 10 hours old, were placed in two small hives on combs containing both honey and pollen. The hives were kept in separate screened cages. A fertile queen was added to each colony. Six days later the colonies were transferred to other hives containing pollen-free combs and the bees were fed only sugar solution.

In order to find out what changes in the thiamine content occur in the newly emerged bees fed a pure carbohydrate diet, a third colony was completely deprived of pollen and the bees received only sugar solution during the entire experimental period.

Weekly samples were taken for thiamine determination. The method of Hennessy and Ceresedo (1939) was used.

The results of the analyses are presented in Table 1.

From Table 1 it is evident that there was a considerable reduction in the thiamine content of the parts of bees which were investigated. At the time of the last determination the bees were 78 to 81 days old and during the 72 days of the pure carbohydrate diet the thiamine loss per bee was 71.4 to 78.3 percent in the heads, 73.7 to 81.8 percent in the abdomens and 41 to 51.5 percent in the thoraces. Similar losses had been observed previously in bees fed a pure carbohydrate diet as to the relation of dry weight and total nitrogen content.

Haydak (1937) kept bees (44 to 47 days old) on a pure carbohydrate diet for 189 days. Before they were placed on this diet the bees had

[1]Paper No. 3140, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2]The author is indebted to Dr. A. G. Richards for helpful suggestions and the critical review of the manuscript.

already reared one cycle of brood. Another cycle was reared while they were fed the sugar solution only. At the end of the experiment the bees had lost 16.2 percent dry weight of their heads, 72.5 percent dry weight of the abdomens and 3.7 percent dry weight of the thoraces. The total nitrogen loss in the heads, abdomens and the thoraces was 36.6, 44.7 and 12.4 percent respectively. As in the case of thiamine, the greatest loss was in the heads and the abdomens. This could have been expected, because both the heads and the abdomens contain some glandular tissues which, in general, have a higher vitamin content than the muscular tissues (Haydak and Vivino, 1943). Moreover, the fat bodies of the abdomens which serve as storage places for nutritive materials, are also likely to lose more thiamine than the thoraces, which contain mostly muscular tissues.

Haydak (1937) kept newly emerged bees on a pure carbohydrate diet and found that when these bees were 59 to 70 days old the dry weight loss was 18.5 percent in the heads, 9.4 per cent in the thoraces and none in the abdomens. The total nitrogen loss was 29.5 percent in the heads, 30 percent in the abdomens and 6.8 percent in the thoraces. The present experiment (Table I, Colony III) shows that in 31 days of the diet the newly emerged bees lost 57.1 percent of the thiamine of their heads, 50 percent of that of their abdomens and 55 percent of that of their thoraces.

During the course of the experiment, one of the colonies on a pure carbohydrate diet (colony II) started rearing brood. The comb with sealed larvae was taken to a constant temperature chamber and the resulting bees, reared by the colony on a pure carbohydrate diet, were analyzed for their thiamine content. The results of the analysis are presented in Table 2.

Haydak (1935) observed that the average total nitrogen content of the abdomens and the thoraces of newly emerged bees reared by a colony fed only sugar solution was lower (18.9 and 0.9 percent less, respectively) than that of those reared under normal conditions. The present study shows that the thiamine content of such bees is also considerably lower than that of normally reared bees (57.1 percent less in the heads, 37.5 percent less in the abdomens and 66.6 percent less in the thoraces).

It is of interest to note that the abdomens of the bees in colony II, which reared brood, had at the start of the pure carbohydrate diet about twice as much thiamine as those of colony I, which did not rear brood. Whether this difference had any relation to the brood rearing activity of colony II could be substantiated only by further experimentation.

The fact that the bees could subsist on a pure sugar diet for a considerable period of time indicates a great adaptability to adverse conditions. Probably, while in growing larval stadia, the bees store body protein and thiamine considerably in excess of their needs and these are drawn upon in time of emergency. Moreover, this storage is augmented during the first week of their adult life in the colony while the bees are occupied by house duties. Haydak (1934) demonstrated that in normal colonies the total nitrogen content of five-day old bees has increased 92.6 percent in their heads, 76 percent in the

TABLE I

CHANGES IN THIAMINE CONTENT OF ADULT WORKER BEES ON PROTEIN-FREE DIET

Parts of Bees	Colony I					Colony II					Colony III				
	Date	Thiamine per Bee	Percent Lost	Thiamine per gm. of Fresh Matter	Percent Lost	Date	Thiamine per Bee	Percent Lost	Thiamine per gm. of Fresh Matter	Percent Lost	Date	Thiamine per Bee	Percent Lost	Thiamine per gm. of Fresh Matter	Percent Lost
		micro-gm.		micro-gm.			micro-gm.		micro-gm.			micro-gm.		micro-gm.	
Heads	8.2	.23		19.6		8.2	.21		15.5		9.2	.07		6.9	
	8.9	.16	30.4	12.8	34.7	8.9	.16	23.8	11.8	23.9	9.14	.04	42.9	3.6	47.8
	8.30	.07	69.6	6.1	68.9	8.16	.15	28.6	12.1	21.9	9.21	.02	71.4	2.2	68.1
	9.28	.06	73.9	6.1	68.9	8.30	.12	42.9	8.9	42.6	9.28	.03	57.1	3.1	55.1
	10.13	.05	78.3	5.2	73.5	10.18	.06	71.4	6.0	61.3					
Abdomens (without digestive tracts)	8.2	.19		14.4		8.2	.33		24.9		9.2	.08		6.8	
	8.9	.12	36.8	11.6	19.4	8.9	.22	33.3	17.2	30.9	9.14	.03	62.5	2.4	64.7
	9.28	.08	57.9	8.8	38.8						9.21	.05	37.5	6.1	10.3
	10.13	.05	73.7	5.4	62.5	10.18	.05	81.8	4.4	82.3	9.28	.04	50.0	4.8	29.4
Thoraces	8.2	.66		16.5		8.2	78		18.8		9.2	.51		13.2	
	8.9	.58	12.1	14.5	12.1	8.9	.65	16.6	15.7	16.5	9.14	.32	37.3	8.2	37.9
	8.30	.41	37.9	10.0	39.4	8.16	.46	41.0	10.9	42.0	9.21	.26	49.0	6.7	49.2
	9.28	.39	40.9	9.5	42.4	8.30	.55	29.5	10.2	45.7	9.28	.23	55.0	5.9	55.3
	10.13	.32	51.5	8.1	50.9	10.18	.46	41.0	11.1	41.0					

abdomens and 37 percent in their thoraces as compared with the same parts of newly emerged bees. However, it should be pointed out that a pure sugar diet adversely affects newly emerged bees.

Haydak (1937a) showed that a colony consisting of newly emerged bees fed for 21 days a pure carbohydrate diet had a 59.9 percent mortality while the pollen-fed control colony during the same period had only 12.7 percent mortality. Toward the end of the experiment the

TABLE II

THIAMINE CONTENT OF NEWLY EMERGED BEES

	Heads		Abdomens (without digestive tracts)		Thoraces		Whole Bees (computed)	
	per bee	per gm. fresh matter	per bee	per gm. fresh matter	per bee	per gm. fresh matter	per bee	per gm. fresh matter
	mcg.	mcg.	mcg.	mcg.	mcg.	mcg.	mcg.	mcg.
Emerged bees from a normal colony	.07	6.9	.08	6.8	.51	13.2	.66	9.0
Emerged bees from a colony on pure carbohydrate diet	.03	2.5	.05	3.2	.17	4.6	.25	3.4
Difference	.04	4.4	.03	3.6	.34	8.6	.41	5.6
Percent less	57.1	63.8	37.5	52.9	66.6	65.2	62.1	62.2

sugar-fed bees also exhibited a marked lack of vitality, remaining motionless upon the comb. Newly emerged bees after 18 days on a sugar diet were given a comb with pollen. Six days later the dry weight of the heads, abdomens and thoraces had increased by 37.5, 20.7 and 5.6 percent respectively. The total nitrogen increase was still greater, being 70, 66.7 and 21.9 percent for the heads, abdomens and thoraces, respectively.

Of course, honey has some protein compounds, so a pure honey diet would not be so depleting as a pure sugar diet. Some indication of this can be gained from Haydak's (1933) experiments. A colony of newly emerged bees was fed a honey and rye flour mixture. Rye flour is a poor source of protein for bees. Sixty-one days later the percent of increase in the total nitrogen was only 3.5 percent in the heads and 12.4 percent in the thoraces. After the diet was changed to fresh whole milk the increase (at 109 days of age) was 21.4 and 30.6 percent for the heads and thoraces respectively.

When newly emerged bees kept on a sugar diet are allowed a normal diet they start brood rearing normally. According to Haydak (1937a) in a colony of newly emerged bees kept for 30 days on a pure sugar diet no attempt at brood rearing was made, in spite of the fact that the queen was laying at the start of the experiment, although later she stopped her egg laying activity. Four days after a comb with pollen and honey was given to this colony, the queen again began to lay eggs. Although only 700 bees were left in the colony, they started brood rearing normally. The resulting bees, found to have 1.85 mg. nitrogen per individual, compared favorably with those reared in the control colony.

For normal life of a colony bees need a balanced diet. For individual maintenance a bee apparently can draw upon food materials present in her body, provided sugar is supplied to her in adequate amounts.

SUMMARY

Two colonies consisting of six to nine day-old bees kept on a normal diet of pollen and honey were transferred to pollen-free comb and fed sugar solution only. The changes in the thiamine content of their heads, abdomens and thoraces were followed. During the 73–77 days of the experimental diet, the bees lost 71 to 78 percent of the thiamine of their heads, 73–82 percent of that of their abdomens and 41–51 percent of that of their thoraces.

A colony consisting of newly emerged bees was kept on a pure sugar diet. The bees in this colony lost about 50 percent of their thiamine during 26 days of the experimental diet.

Newly emerged bees reared by the colony fed pure sugar diet had 50–60 percent less thiamine in their bodies than did similar bees reared under normal conditions.

For the normal life of the colony bees need a balanced diet. For individual maintenance bees apparently can draw upon the food materials present in their bodies, provided sugar is supplied to them in adequate amounts.

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A POLYHEDROSIS VIRUS OF THE IMPORTED CABBAGEWORM AND ITS RELATION TO A POLYHEDROSIS VIRUS OF THE ALFALFA CATERPILLAR[1,2]

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In July, 1951, the writer found larvae of the imported cabbageworm, *Pieris rapae* (L.), infected with a polyhedrosis virus in the laboratory. This appears to be the first record of such a virus in *Pieris rapae*. Some workers have reported this species to be susceptible to virus diseases (Glaser, 1928; Sweetman, 1936; Bergold, 1943; Steinhaus, 1946, 1949), but there is no positive record of a polyhedrosis virus attacking it. Steinhaus (1949) has stated, "The imported cabbageworm, *Pieris rapae* (Linn.), apparently suffers from a polyhedrosis, but the actual demonstration of polyhedra does not seem to have been reported". He suggested the presence of a polyhedrosis in the imported cabbageworm because of several cases of "wilt" reported from this species. The symptom of "wilt," which is an important characteristic of most polyhedroses, was reported by Richards (1940) and Holdaway and Lucas (1941) in *P. rapae* larvae infected with apparent virus disease. These cases of "wilt" in infected larvae of *Pieris rapae* were probably caused by the granulosis virus, *Bergoldia virulenta* Tanada, which produces typical symptoms of "wilt" in the cabbageworm (Tanada, 1953). Moreover, some of the early records of "bacterial diseases" (Forbes, 1886; Wilson, Pickett, and Gentner, 1919; Brown, 1930), which resulted in the wilting of the imported cabbageworm, were probably caused by the granulosis virus.

EXPERIMENTAL PROCEDURE

The imported cabbageworms were reared in the laboratory of the University of California Agricultural Experiment Station in Albany, California. The temperature in the laboratory was usually between 21 to 26° C but occasionally reached extremes of 17 and 30° C. The daily relative humidity usually ranged from 50–60%, but occasionally dropped to 18% or rose to 74%.

[1]Portion of a thesis submitted as a partial fulfillment for the degree of Doctor of Philosophy from the University of California, Berkeley. The study was conducted at the Laboratory of Insect Pathology, Department of Biological Control, University of California, Berkeley.

I wish to express grateful appreciation: to Dr. Edward A. Steinhaus for his friendly guidance and assistance throughout the work, to Dr. Clarence G. Thompson for making available the laboratory facilities at Albany and for supplying the various pathogens and insect hosts, to Mr. Kenneth M. Hughes for his generous assistance in certain of the techniques used in insect pathology, and to the other members of the Laboratory of Insect Pathology (University of California, Berkeley) for the frequent help rendered during the study.

[2]Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Technical Paper No. 294.

The method of rearing the cabbageworms was the same as that described for a previous study (Tanada, 1953). The cabbageworms were fed with the following cruciferous plants: the chicken kale or thousand-headed kale, *Brassica oleraceae* var. *acephala* DC., and two species of wild mustard, *B. campestris* L. and *B. Kaber* (DC.)Wheeler (= *B. arvensis* sensu Rabenh.)[3].

Other insect species which were used in the experiments were kindly supplied either as eggs or very young larvae by Dr. C. G. Thompson, assistant entomologist at the University of California, Berkeley. All of these species were fed alfalfa, *Medicago sativa L.*, or bur clover, *M. hispida* Gaertn., except for the California oakworm, which was fed leaves of the California live oak, *Quercus agrifolia* Née.

The pathogens were suspended in a 0.2% blood albumin solution and leaves dipped into this solution were fed to the larvae. In both the treated and control groups, the larvae were reared individually in sterile containers. Larvae that died were dissected and the body contents examined under the microscope to determine the nature of the pathogen.

For the histopathological study, the larvae were fixed in alcoholic Bouin's solution (Duboscq-Brasil's modification) and prepared for sectioning by dehydration through a methyl benzoate and benzene series, and thence into paraffin (see Romeis, 1948).

The virus specimens for electron microscopy were prepared and shadowed with palladium according to the technique described by Steinhaus, Hughes, and Wasser (1949). The virus was observed through the RCA electron microscope, type EMU-2B. The size of the specimens was calculated from polystyrene particles that were 260 millimicrons in diameter.

PATHOLOGY

The infectivity of the polyhedrosis virus to the imported cabbageworm was investigated in nine separate tests. In each test, 10 to 20 larvae were fed the virus, and approximately the same number of larvae was used as controls. In all, 124 larvae were treated and 119 larvae left untreated as controls. At least three or more of the treated larvae died of polyhedrosis in eight of the nine tests. In the ninth test, none died from polyhedrosis. Out of the 124 larvae which were fed the virus, 80 or 64.5% died from polyhedrosis, while none of the 119 control larvae died from it. Thus the polyhedrosis virus was infectious when fed to the imported cabbageworm, but was of low virulence.

The larvae began to show symptoms about 7 days after feeding on the virus. As the infection from polyhedrosis advanced, the larvae became lighter in color and appeared mottled. They increased in size and were often distinctly bloated. At an advanced stage of infection, the larvae gradually became lethargic, lost their appetite, and stopped feeding 2 or 3 days before death. Prior to death, the bloated larvae decreased in size. They were mottled with light yellow and

[3]The scientific names of the plants were obtained from Abrams (1940) and Bailey (1949).

green, or the dorsum turned light reddish brown, but in either case the venter was yellowish white. Occasionally there were a few dark spots on the integument. The larvae sometimes clung to the sides of the containers by their abdominal prolegs with the anterior portion hanging, but usually they lay inactive on the bottom of the container. The diseased specimens sometimes discharged a dark fluid from their mouth or anus, or from a rupture of their delicate integument. The dark fluid became viscid and occasionally fastened the larvae to the substratum. Some of the larvae were diarrheic. At death, the integument generally became fragile and easily torn. The body fluid was milky to brownish and somewhat viscous. The dead larvae darkened quickly.

If the infected larvae matured to the prepupal stage, they sometimes formed abnormal prepupae which darkened and dried to shriveled, rather brittle, remains. If the larvae pupated, they often died and turned dark brown to black. Although the tissues had mainly decomposed to a dark fluid, the pupae never wilted because of their rigid exoskeleton.

Rarely, a larva died shortly after showing the first signs of color change. Its body contents rapidly decomposed into a fluid mass. The larval integument was very fragile and the larva broke down into a wilted condition. These symptoms are more typical of the polyhedrosis of other insects.

The first and third instar larvae when infected with the polyhedrosis virus died in 11 to 17 days. When the larvae were infected in the fourth and fifth instars, they died in 13 to 21 days. Because of the long period of infection[4] the infected cabbageworms were still in the larval stage even after control larvae of comparable age at the start of the trial had developed into adults.

When infected larvae were dissected, the body fluid was either clear or turbid, depending on the stage of infection. Some of the fat bodies in an infected individual appeared normal but other fat bodies within the same individual were slightly yellow and had white specks or a granular consistency. The opacity of the fat body was not as marked as in the case of granulosis (Tanada, 1953). The digestive tract was usually flaccid, and the mid-gut more or less yellow.

In longitudinal sections of infected cabbageworms, the polyhedra were found most abundantly in the nuclei of cells of the hypodermis, adipose tissue, and tracheal matrix. Some polyhedra were also present in the nuclei of blood cells and of cells lining the Malpighian tubules and digestive tract.

THE CAUSATIVE AGENT

One hundred polyhedra taken at random in a water suspension ranged from 0.7 to 2.7 microns in diameter and averaged 1.4 microns. Occasionally much larger polyhedra, up to 5 microns, were found. Although the polyhedra varied in shape, they were ordinarily five-to eight-sided with more or less angular corners (fig. 1A). The polyhedra

[4]The period of infection may be defined as the interval from the time the host feeds on the pathogen until the death of the host from a frank infection.

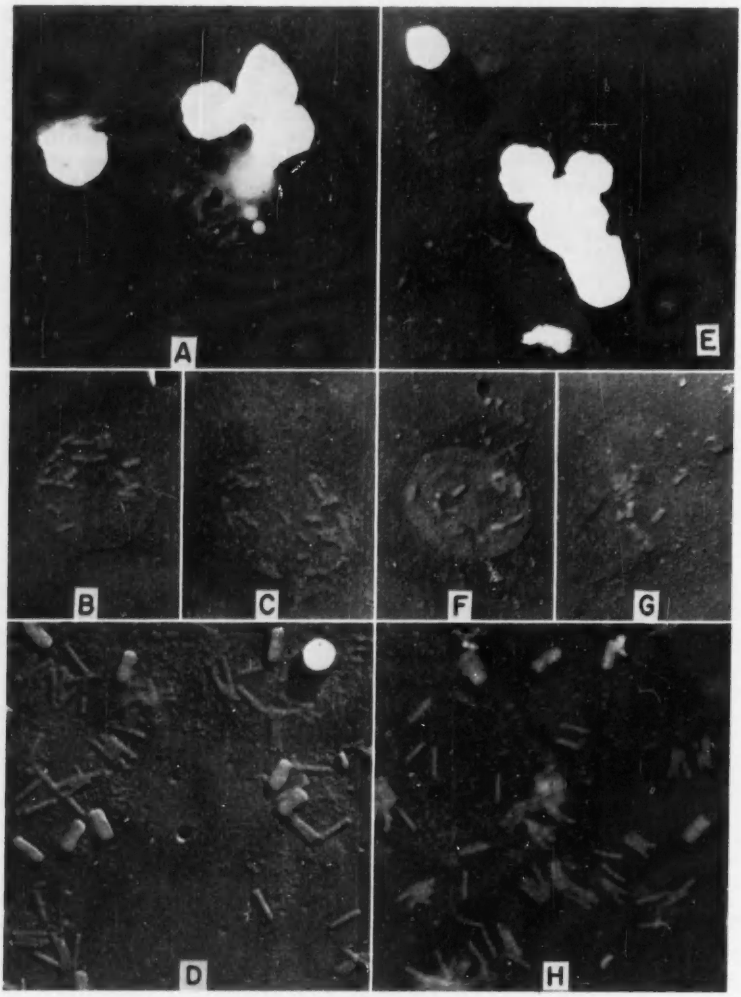

FIG. 1. Electron micrographs of polyhedrosis viruses from the imported cabbageworm (A, B, C, D) and the alfalfa caterpillar (E, F, G, H). A, E. Undissolved polyhedra. B. C. F. G. Partially dissolved polyhedra. D, H. Free virus particles. The white spheres in A and D are polystyrene particles of 260 millimicrons in diameter. Approximate magnifications A, E—8,500 x; B, C, F, G—11,000 x; D, H—20,000 x. H, photographed by K. M. Hughes with an older method of electron microscopy.

were soluble in weak alkali and were not optically active when viewed through polarized light.

When a polyhedron was partially dissolved by weak alkali, bundles of virus particles were found enclosed in a membrane (fig. 1B, C), which was first described by Hughes (1950). In 58 partially dissolved polyhedra, the number of virus bundles ranged from 3 to 19 per polyhedron, with an average number of 9 virus bundles per polyhedron.

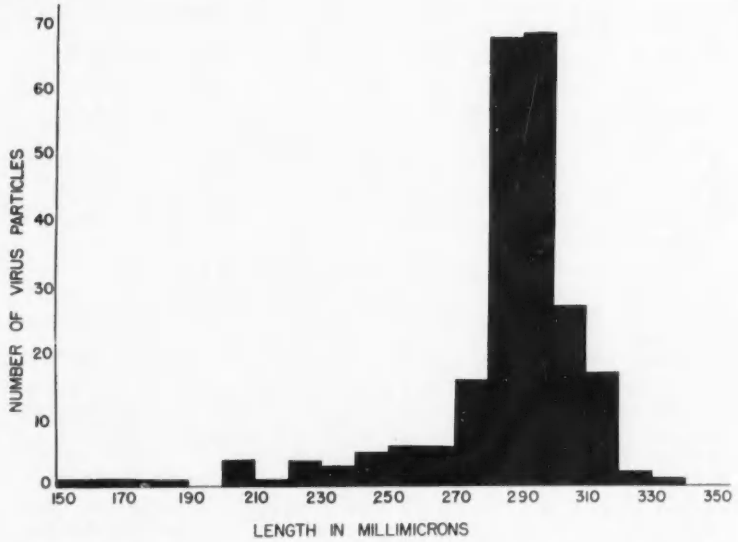

FIG. 2. Distribution in the lengths of 231 virus particles of the polyhedrosis in the imported cabbageworm.

The polyhedrosis virus particles showed great variation in size ranging in width from 41 to 90 millimicrons, with most of them being 51 to 60 millimicrons wide. They were from 151 to 340 millimicrons long, with most of them being 281 to 300 millimicrons (fig. 2). The extremely short rods were not artifacts, because in partially dissolved polyhedra, short virus bundles were found together with long virus bundles (fig. 1C). Each virus bundle contained approximately two to four virus particles.

Attempts were made to transmit the polyhedrosis virus of *Pieris rapae* to the beet armyworm, *Laphygma exigua* (Hbn.), and the California oakworm, *Phryganidia californica* Pack. In each trial, the virus inoculum was tested for its virulence on 5 to 10 cabbageworms, and an equal number of untreated larvae served as controls. The virus inoculum was always infectious to the cabbageworms. Fifteen larvae of *L. exigua* and 20 of *P. californica* were fed the virus inoculum and an

equal number of each species was kept as controls. None of the larvae of *L. exigua* or *P. californica* died from polyhedrosis, and all reached adulthood.

The polyhedrosis viruses of the California oakworm, the beet armyworm, and the western yellow-striped armyworm, *Prodenia praefica* Grote, were fed to 20, 15, and 20 cabbageworms respectively. An equivalent number of cabbageworms served as controls. None of the treated and control larvae died from polyhedrosis.

The cross infectivity tests between the polyhedrosis virus of the imported cabbageworm and that of the alfalfa caterpillar with their respective hosts indicated that the two viruses were infectious to both hosts. This study is discussed in detail in the following section.

Identity of *Pieris* and *Colias* Polyhedroses

In the first test, the virus inoculum was prepared from the blood of an imported cabbageworm infected with polyhedrosis. The external surface of the infected cabbageworm was disinfected by immersing the larva for 5 minutes in a tincture of merthiolate. The larva was washed with 12 rinses of sterile distilled water. The last two rinses of water were saved for one of the treatments. An abdominal proleg of the disinfected larva was cut off and three to four drops of blood were collected with a sterile medicine dropper and diluted with about 15 ml. of a 0.2% blood albumin solution. Under the microscope, the inoculum was found to contain polyhedra.

Sixteen alfalfa caterpillars were fed the virus inoculum. The control group of 20 larvae was divided into three parts: (1) ten larvae were fed the last two rinses of water which were used to wash the merthiolate off the disinfected larva; (2) five larvae were fed the blood albumin solution; (3) five larvae were untreated.

Fifteen of the 16 larvae which were fed the virus died from polyhedrosis in 10 to 12 days and one died from an unknown cause. None of the control group died from polyhedrosis, but two died from bacterial infections. Since none of the 10 larvae which were fed the wash water died from polyhedrosis, there was little likelihood that the virus inoculum was contaminated by virus present on the external surface of the cabbageworm.

In the second test, the inoculum was prepared from two cabbageworms which had died from polyhedrosis. The larvae were macerated and the polyhedra were concentrated by repeated washing and centrifugation. The sediment of polyhedra was dried for 3 days at room temperature in order to destroy most of the nonspore forming bacteria. Half of the dried sediment was suspended in 50 ml. of 0.2% blood albumin solution.

Twenty-five alfalfa caterpillars were fed the virus inoculum, 15 were fed the blood albumin solution, and 10 were untreated. All 25 larvae which were fed the virus died from polyhedrosis in 9 to 13 days. Seven of the 25 control larvae died: three from polyhedrosis, one from bacterial infection, and three from an amicrobic cause.

In the third test, the external surface of the cabbageworm was disinfected with merthiolate and the blood was extracted as in Test I. Under the microscope many polyhedra were present in the infected

blood. The blood was dried overnight to destroy most of the non-spore forming bacteria in the blood. The dried blood was diluted with about 15 ml. of 0.2% blood albumin solution. There were 15 alfalfa caterpillars in both the treated and the control groups. The control group was separated into one portion of 10 larvae which were fed the blood albumin solution and another portion of 5 untreated larvae.

Fourteen of the 15 treated larvae died of polyhedrosis in from 7 to 9 days, and one larva died of bacterial infection in 4 days. Two of the control larvae died from bacterial infection and one from a fungus infection.

TABLE I

SUSCEPTIBILITY OF THE ALFALFA CATERPILLAR TO THE POLYHEDROSIS VIRUS OF THE IMPORTED CABBAGEWORM

TEST	TREATMENTS	NUMBER LARVAE TESTED	TOTAL DEAD INDIVIDUALS		TOTAL ADULTS
			POLYHEDROSIS	OTHERS	
I	Control 1	5	0	0	5
	Control 2, Blood Albumin	5	0	1	4
	Control 3, Wash Water	10	0	1	9
	Virus Suspension, Fresh Infected Blood	16	15	1	0
II	Control 1	10	1	2	7
	Control 2, Blood Albumin	15	2	2	11
	Virus Suspension, Dried Polyhedra	25	25	0	0
III	Control 1	5	0	1	4
	Control 2, Blood Albumin	10	0	2	8
	Virus Suspension, Dried Infected Blood	15	14	1	0

The results of the above three tests (Table I) indicated that the *Pieris* polyhedrosis was transmissible to the alfalfa carterpillar, which developed symptoms characteristic of its own polyhedrosis. However, the transmission to the alfalfa caterpillar occasionally failed apparently because of the presence of bacterial "opportunists"[5] in the virus inoculum which killed the larva before the virus could manifest itself.

[5]Steinhaus (1949) defined "opportunists" as those microorganisms which live in constant association with the host, such as those in the alimentary tract, and which, under certain conditions, such as when the resistance of the host is lowered, invade the more susceptible parts of the insect's body.

Since the polyhedrosis virus of the imported cabbageworm was infectious to the alfalfa caterpillar, the question occurred—would the polyhedrosis virus, *Borrelina campeoles* Steinhaus, of the alfalfa caterpillar also infect the imported cabbageworm? The transmission of *Borrelina campeoles* to the imported cabbageworm was investigated in two tests. In the first test, 20 third instar cabbageworms were fed the *Colias* polyhedrosis virus and 20 served as controls. Nineteen of the 20 treated larvae died from polyhedrosis. Twelve of the 19 died in the larval stage in 10 to 18 days after infection and 7 died in the pupal stage.

In the second test, 20 cabbageworms were fed the virus, and 19 were used as controls. The control larvae were divided into one group of 10 which were fed the blood albumin solution and another group of nine which were untreated. Sixteen of the 20 treated cabbageworms died of polyhedrosis, eight as larvae and eight as pupae. The larvae died in 16 to 19 days. In the control group, four larvae died from causes other than polyhedrosis.

TABLE II

SUSCEPTIBILITY OF THE IMPORTED CABBAGEWORM TO THE POLYHEDROSIS VIRUS OF THE ALFALFA CATERPILLAR (The results of two trials)

TREATMENTS	NUMBER LARVAE TESTED	NUMBER DEAD LARVAE		NUMBER DEAD PUPAE		TOTAL DEAD POLYHEDROSIS	TOTAL ADULTS
		POLYHEDROSIS	OTHERS	POLYHEDROSIS	OTHERS		
Control 1	29	0	1	0	0	0	28
Control 2, Blood Albumin	10	0	3	0	0	0	7
Virus Suspension	40	20	1	15	0	35	4

The combined results of the two tests are presented in Table II. The cabbageworms infected with *Colias* polyhedrosis virus showed symptoms similar to those resulting from the *Pieris* polyhedrosis virus.

Since the above transmission studies indicated that the virus of each of the two insect species was infectious to the other host insect, a further study was undertaken to determine if the virus would reinfect the original host after passage through the other insect species. The experiment with the polyhedrosis virus of *Pieris rapae* was performed twice. In the first replicate, the virus inoculum was prepared from an alfalfa caterpillar which had been infected 7 days previously with *Pieris* polyhedrosis and which showed distinct signs of infection. In the second replicate, the alfalfa caterpillar was in a more advanced stage of polyhedrosis than that of the first replicate. Both larvae were immersed in a tincture of merthiolate for 5 minutes and washed with 12 rinses of sterile distilled water. The last two rinses were saved for one of the treatments.

There were two types of inocula, one composed of infected blood and the other of infected solid tissues, such as skin, muscle, and fat. The blood was extracted from the prolegs. The solid tissues were removed by cutting a portion of the skin, fat, and muscle (but not the gut) from the disinfected larva. The solid tissues were macerated in a sterile mortar. Both the blood and the macerated solid tissues were diluted with 0.2% blood albumin solution. The treatments consisted of: (1) a treated group which was fed the virus inoculum made from the infected blood; (2) a treated group which was fed the virus inoculum made from infected solid tissues; (3) a control group which was fed the wash water; (4) a control group which was fed the blood albumin solution; (5) a control group of untreated larvae. Cabbageworms in their third instar were used in both replicates.

TABLE III

REINFECTION OF THE IMPORTED CABBAGEWORM WITH ITS POLYHEDROSIS VIRUS AFTER PASSAGE THROUGH THE ALFALFA CATERPILLAR (The results of two replicates)

TREATMENTS	NUMBER LARVAE TESTED	*Number Dead Larvae*		*Number Dead Pupae*		TOTAL DEAD POLYHEDROSIS	TOTAL ADULTS
		POLYHEDROSIS	OTHERS	POLYHEDROSIS	OTHERS		
Control 1.....	10	0	0	0	1	0	9
Control 2, Blood Albumin.....	10	0	0	0	2	0	8
Control 3, Wash Water.....	20	0	0	0	0	0	20*
Virus Suspension, Infected Blood	20	7	0	1	0	8	12**
Virus Suspension, Infected Solid Tissues.....	20	12	0	7	0	19	1

*Three normal hibernating pupae.
**One normal hibernating pupa.

The results of the two replicates are summarized in Table III. Eight of the 20 treated larvae, which were fed fresh infected blood, died from polyhedrosis. Of the 20 treated larvae which were fed infected solid tissues, 19 died from polyhedrosis. The control group of 40 individuals had three pupae which died from an amicrobic cause. Since the group treated with wash water had no mortality, the two virus inocula must not have been contaminated with virus present on the surfaces of the alfalfa caterpillars.

There were three separate attempts to reinfect the alfalfa caterpillars with its polyhedrosis virus after passage through the imported cabbageworm. The cabbageworms, which were infected with *Colias* polyhedrosis, were disinfected with tincture of merthiolate and rinsed with sterile distilled water. The last three rinses of wash water were

kept for use in one of the treatments. In the first test, about one-fourth of the body wall of a disinfected cabbageworm was removed without rupturing the digestive tract. The tissues were ground in a sterile mortar and diluted with 50 ml. of a 0.2% blood albumin solution. In the second and third tests, the virus inocula were prepared from 3 to 4 drops of infected blood that were obtained from the prolegs of a disinfected cabbageworm. The blood was diluted with 20 ml. of 0.2% blood albumin solution. The virus inocula of both tests, when examined under the microscope, contained many polyhedra.

In each of the three tests, 10 alfalfa caterpillars were fed the virus inoculum. The controls were separated into three groups: the first group was fed the wash water, the second group was fed the blood albumin solution, and the third group was untreated.

TABLE IV

REINFECTION OF THE ALFALFA CATERPILLAR WITH ITS POLYHEDROSIS VIRUS AFTER PASSAGE THROUGH THE IMPORTED CABBAGEWORM

TEST	TREATMENTS	NUMBER LARVAE TESTED	*Total Dead Individuals*		TOTAL ADULTS
			POLYHEDROSIS	OTHERS	
I	Control 1	5	1	1	3
	Control 2, Blood Albumin	5	0	0	5
	Control 3, Wash Water	10	0	0	10
	Virus Suspension	10	10	0	0
II	Control 1	5	0	2	3
	Control 2, Blood Albumin	5	0	2	3
	Control 3, Wash Water	10	1	2	7
	Virus Suspension	10	10	0	0
III	Control 1	7	0	2	5
	Control 2, Blood Albumin	7	0	1	6
	Control 3, Wash Water	10	0	3	7
	Virus Suspension	10	10	0	0

The results are given in Table IV. All of the alfalfa caterpillars which were fed the virus inocula died in 4 to 10 days with symptoms typical of the *Colias* polyhedrosis. Among the controls, two larvae died of polyhedrosis, one in the untreated group, the other in the group treated with wash water. Since only one of the 30 larvae which were fed the wash water died of polyhedrosis, the virus inocula were apparently not contaminated by virus present on the external surface of the cabbageworms. These tests gave further evidence that the

polyhedrosis viruses of *P. rapae* and *C. philodice eurytheme* were apparently identical.

Serological Study

The relationship between the polyhedrosis of *Pieris rapae* and that of *Colias philodice eurytheme* was investigated serologically. The antigen of the *Pieris* polyhedra was prepared from several cabbageworms which had died of polyhedrosis. The cabbageworms were macerated in a sterile Waring blender, and the suspension of tissues was filtered through a coarse filter paper to remove the large particles. The polyhedra in the filtrate were purified by centrifugation at a low speed and were washed five times with sterile 0.8% physiological salt solution. The antigen of *Colias* polyhedra was obtained from 1 to 2 ml. of the concentrated sediment of polyhedra in the bottom of a flask containing decomposed alfalfa caterpillars. The *Colias* polyhedra were purified in the same manner as the *Pieris* polyhedra. The antigen of *Pieris* polyhedra contained about 3,000 polyhedra per cubic millimeter and that of *Colias* polyhedra about 51,000 polyhedra per cubic millimeter. Each antigen was diluted with an equal volume of neutral glycerine and stored in the refrigerator.

Each of the antigen suspensions was injected into a three months old rabbit. Prior to the inoculation, approximately 5 to 10 ml. of blood were collected from the ear veins of each rabbit for the titre of antibody in normal serum. The antigens were injected into the rabbits at 5 days interval according to the following protocol:

Injection No.	Amount Injected (ml.)	Mode of Injection
1	2	Subcutaneous
2	4	Subcutaneous
3	0.5	Intraperitoneal
4	1	Intraperitoneal
5	1.5	Intraperitoneal
6	0.5	Intravenous
7	1.5	Intravenous
8	3	Intravenous

The subcutaneous injection was made slightly lateral to the middorsal line and about a third of the distance from the hind to the fore legs. The intraperitoneal inoculation was performed in the inguinal region, and the intravenous injection was in the ear vein.

The rabbits were killed and blood was collected from the heart 9 days after the last injection. After standing in the refrigerator overnight, the clear serum which had separated from the blood clot was pipetted into sterile test tubes. Additional serum was obtained by centrifuging the blood clot for 10 to 15 minutes at about 1500 rpm.

The agglutination test was carried out with a *Pieris* antigen which contained about 27,000 polyhedra per cubic millimeter and a *Colias* antigen which had about 29,000 polyhedra per cubic millimeter. About 0.5 ml. of diluted antiserum and 0.5 ml. of antigen were used in the agglutination test. After titration, the agglutination tubes were incubated in a water bath at 37° C for 2 hours before reading. The

TABLE V

THE AGGLUTINATION OF *Pieris* AND *Colias* ANTIGENS BY THEIR RESPECTIVE ANTISERA

Antisera	Antigens	Dilutions of Antiserum										Control Saline
		1: 100	1: 200	1: 400	1: 600	1: 800	1: 1200	1: 1600	1: 2400	1: 3200	1: 6400	
Pieris	*Pieris*	++++	++++	++++	+++	++	+	+	−	−	−	−
	Colias	++++	++++	+++	++	+	−	−	−	−	−	
Colias	*Pieris*	++++	++++	++++	++++	++++	++++	++++	++	++	+	−
	Colias	++++	++++	++++	++++	++++	++++	+++	++	+	−	

tubes were stored overnight in a refrigerator and read again within 24 hours. The end point of titration was designated as the 50% combination (++).

The results are presented in Table V. The titre for normal antiserum was about 1:40. Both the *Pieris* and *Colias* antigens gave positive reactions at high titres with each antiserum. With the *Pieris* antiserum, the highest titre was 1:800 with *Pieris* antigen and 1:600 with *Colias* antigen. The *Colias* antiserum appeared stronger than the *Pieris* antiserum. With the *Colias* antiserum, the *Colias* antigen had the highest titre at 1:2400 and the *Pieris* antigen at 1:3200.

The antigenic properties of polyhedra from three other insect species were compared with those of the polyhedra from *Colias philodice eurytheme* and *Pieris rapae*. The antigen of *Phryganidia californica* had about 21,000 polyhedra per cubic millimeter, that of *Laphygma exigua* about 13,000 polyhedra per cubic millimeter, and that of the tent caterpillar, *Malacosoma* sp., about 23,000 polyhedra per cubic millimeter. A solution of granules of *Bergoldia virulenta*, the granulosis virus of *Pieris rapae*, was also included in the tests.

TABLE VI

THE AGGLUTINATION TITRES OF VARIOUS ANTIGENS WITH THE *Pieris* AND *Colias* ANTISERA

ANTISERA	TYPES OF ANTIGEN					*B. virulenta* Granules
	Colias	*Pieris*	*Phryganidia*	*Laphygma*	*Malacosoma*	
Pieris	1:600	1:800	<1:100	1:200	1:100	1:400
Colias	1:2400	1:3200	1:100	1:200	1:200	1:400

The results of these tests, together with part of those from the previous test, are given in Table VI. None of the antigens from the above three species was agglutinated in a dilution greater than 1:200 in either the *Pieris* or the *Colias* antiserum. Once again, the titres of the *Pieris* antiserum were lower than those of the *Colias* antiserum. These results indicated that the polyhedra of *Pieris rapae* and *Colias philodice eurytheme* were much more closely related to each other than to those of the other species. Since the polyhedra from the three different hosts showed some cross-reactions, this fact might indicate a slight relationship with the polyhedra of *Pieris rapae* and *Colias philodice eurytheme*.

Surprisingly, the antigen composed of the granules of *Bergoldia virulenta* was agglutinated to a titre of 1:400 with both antisera. Although more extensive studies are required to establish the antigenic relationship between granules and polyhedra, these results may in-

TABLE VII

THE PRECIPITATION OF *Pieris* AND *Colias* ANTIGENS (Fraction B) BY THEIR RESPECTIVE ANTISERA

ANTISERA	ANTIGENS	DILUTIONS OF ANTISERUM								CONTROL SALINE
		1:25	1:50	1:75	1:100	1:150	1:200	1:250	1:300	
Pieris	*Pieris*	++++	++++	+++	+++	+++	++	+	−	−
	Colias	++++	++++	++++	+++	++	+	−	−	
	Alkali	−	−	−	−	−	−	−	−	
Colias	*Pieris*	++++	++++	++++	++++	++++	++++	++++	+++	−
	Colias	++++	++++	++++	++++	++++	++++	+++	+	
	Alkali	−	−	−	−	−	−	−	−	

dicate that the relationship of viruses, as determined serologically, is not necessarily associated with the type of inclusion bodies formed by the virus, but is more closely associated with the host species in which the inclusion bodies are developed.

In addition to the agglutination test, a precipitation test was conducted with the *Pieris* and *Colias* antisera. The antigens of the two polyhedroses were prepared from their polyhedra which were purified by centrifugation as described previously. The sediment of polyhedra was treated for about 5 hours with 10 ml. of equal parts of 0.2 M sodium carbonate and 0.1 M sodium chloride. The large undissolved particles were separated by centrifugation at 5,000 rpm. for 10 minutes. This sediment was washed once with about 10 ml. of 0.8% physiological saline and designated as Fraction A.

The initial supernatant, that had been put into solution with the alkali, was centrifuged at 13,000 rpm. for 45 minutes. The supernatant from this treatment was desginated as Fraction B. The sediment was resuspended in 10 ml. of 0.8% physiological saline and centrifuged for 1¼ hours at 13,000 rpm. The final sediment was diluted with 10 ml. of physiological saline and labeled Fraction C. When a sample of Fraction C was examined under the electron microscope, it was found to contain many virus particles.

The pH of the three fractions and that of the alkali solution, 0.2 M sodium carbonate plus 0.1 M sodium chloride, were adjusted with dilute hydrochloric acid to a reaction between pH 7 and 8. A Beckman's glass electrode pH meter was used to determine the pH of the solutions. The precipitation tubes were made from soft glass tubing of about 0.7 mm. internal diameter. With a capillary pipette about 0.1 ml. of diluted antiserum, in each of the different test dilutions, was layered beneath 0.5 ml. of the test antigen. The tubes were read about 30 to 45 minutes later.

Neither Fraction A, which was composed of undissolved large particles, nor Fraction C, which contained free virus particles, gave positive reactions with either of the two antisera. Only Fraction B, the alkali soluble fraction of the polyhedra, gave a positive precipitation test.

The results of the tests with Fraction B are given in Table VII. The normal serum at a dilution of 1:10 showed no reaction when treated with Fraction B. With the *Pieris* antiserum, the highest titre was 1:150 for the *Colias* antigen and 1:200 for the *Pieris* antigen. The antiserum for the *Colias* polyhedra gave positive reactions beyond 1:300 with the *Pieris* antigen and up to 1:250 with the *Colias* antigen. The alkali solution of 0.2 M sodium carbonate and 0.1 M sodium chloride with an adjusted pH of 7 to 8 gave no reaction with either antiserum.

The above results, which confirmed those of the agglutination tests, indicated that the polyhedroses of *Pieris rapae* and *Colias philodice eurytheme* were closely related if not identical. The results would be much more conclusive with the use of more rabbits, additional replications, and other serological tests, but time and circumstance did not permit the extension of the work.

STUDY ON THE LATENT VIRUS INFECTION

In 1951, Vago reported that in virus diseases of insects, the pathogenic agent was present in large majority of healthy individuals in a latent state, a condition of "latentia." Other workers (Bergold, 1951; Smith and Wyckoff, 1951) had also noted a latent condition for the diseases they had investigated.. Vago (1951) listed several factors which favor the activation of the virus from a latent condition. These factors were: (1) certain chemicals, (2) inherent variations among the individuals, (3) certain physical conditions, (4) strong solar radiation, (5) types of food, and (6) stimulation at critical periods in the development of the insects.

The imported cabbageworm was subjected to some of these factors to determine if a latent virus could be activated in them. Rearing the cabbageworms at a high temperature (36° C) and moderate relative humidity (45%) did not result in an increased incidence of either polyhedrosis or granulosis (Tanada, 1953). Vago (1951), however, obtained an increase in virus infections among caterpillars which were raised at high humidity and high temperature. According to Yamafuji and Cho (1947), moderate heating caused silkworms to develop polyhedrosis.

The effect of freezing temperatures was also investigated. In a preliminary test, groups of five fourth instar larvae were treated for 2, 4, 6, and 8 minutes at —32° C. Ten untreated larvae were used as controls. In 24 hours, one larva died after 4 minutes of cold treatment, three larvae died after 6 minutes, and four larvae died after 8 minutes. All the deaths were caused directly by the subfreezing temperature and not by an infectious disease. The remaining larvae, including the control larvae, developed into adults.

In a second test, 20 fourth instar larvae were treated for 4 minutes at —32° C and 10 larvae were untreated. Although the subfreezing temperature so severely shocked the larvae that they vomited fluid and appeared lifeless for a short time, none of them died. Two of the pupae in the control and one in the treated group went into hibernation. The remainder emerged as adults.

The above tests indicated that a short period of exposure to subzero temperature apparently did not activate virus diseases in the cabbageworm.

Certain chemicals and types of food have been reported as causing the development of virus diseases in insects (Paillot, 1943; Yamafuji and Cho, 1947; Yamafuji, Omura, and Yoshihara, 1951; Yamafuji and Yoshihara, 1951; Vago, 1951). Since the proteins of one insect species might induce polyhedrosis in another species, the larvae of *Colias philodice eurytheme* and *Pieris rapae* were fed the body proteins from the healthy larva of the other insect species.

In the first test, two healthy alfalfa caterpillars in their fifth instar were macerated separately in sterile mortars and each diluted with 50 ml. of 0.2% blood albumin solution. One inoculum was fed to 15 cabbageworms and the other to 12 cabbageworms. In the control group, five larvae were fed the blood albumin solution and five were untreated. The cabbageworms were in their fourth instar. None

of the larvae which were fed the healthy body contents of the alfalfa caterpillar died from polyhedrosis, but one died in the pupal stage from bacterial infection. The remainder gave rise to adults except for two pupae which went into hibernation. In the control group, one pupa died from bacterial infection and the others developed into adults.

In the second test, two healthy imported cabbageworms in their fourth instar were prepared separately as inocula in the same manner as above. The inoculum from one larva was fed to 15 alfalfa caterpillars and that from the other larva was fed to 10 alfalfa caterpillars. Ten alfalfa caterpillars were fed the blood albumin solution and five were untreated. None of the 25 alfalfa caterpillars which were fed the body tissues of healthy cabbageworms died from polyhedrosis. However, three died as pupae, one of them from bacteria and the others from an amicrobic cause. The remaining 22 reached adulthood. One of the control larvae died from polyhedrosis but the others developed into adults.

Accordingly, in both *Colias philodice eurytheme* and *Pieris rapae*, the proteins which were obtained from apparently healthy larvae and which were foreign to the inoculated species did not cause the development of polyhedrosis. In addition to the proteins from healthy larvae, large quantities of polyhedra from insect species other than *Colias philodice eurytheme* were fed to the larvae of *Pieris rapae* without causing any polyhedrosis. This has already been discussed under the subject of the transmission of polyhedrosis between *Pieris rapae* and other insect species.

Since potassium nitrite and hydroxylamine were found to stimulate the production of polyhedrosis in the silkworm, *Bombyx mori* L. (Yamafuji and Cho, 1947; Yamafuji, Omura, and Yoshihara, 1951; Yamafuji and Yoshihara, 1951), these chemicals were fed to the imported cabbageworm. In the first test, 15 fourth instar cabbageworms were fed leaves dipped in 5% potassium nitrite on the first day and 2.5% potassium nitrite on the fifth, eighth, ninth, and eleventh days. The concentration of the potassium nitrite was reduced one-half after the first feeding because it scorched the food and the larvae refused to eat the food. The control group consisted of 15 fourth instar larvae.

Six of the treated larvae died on the second and third days. They contained many small particles in their bodies. The particles were observed under the electron microscope and were found to be not identical with those of granulosis. Most probably, the larvae died from poisoning caused by an overdose of potassium nitrite. The remaining nine larvae, which were fed nitrite, did very little feeding on the treated leaves and their growth was greatly retarded as compared with that of the control larvae, but they all matured to adults. All the control larvae developed into adults.

In the second test, 10 fourth instar cabbageworms, which had been first heated at 45° C. for 10 minutes, were fed with mustard leaves dipped in an aqueous solution of hydroxylamine. Ten other larvae were left untreated as controls. The concentration of the hydroxylamine solution was diluted from N/4 to N/8 after the first day since the larvae refused to feed on the leaves treated with the higher concentration. Except for the third day, new food dipped in N/8 hy-

droxylamine was given to the larvae daily until they pupated. Two of the treated larvae died as pupae from an amicrobic cause. The remaining eight treated larvae and those of the control group developed into adults.

The above studies, although conducted with a rather small number of larvae, indicated either that the healthy cabbageworms ordinarily did not have virus in the latent state or that other stimulants besides the ones tested were required to activate the latent virus.

DISCUSSION

Although *Pieris rapae* and *Colias philodice eurytheme* belong to genera which are not closely related phylogenetically (Klots, 1931–1932, 1951; Ford, 1946), they both appear to be susceptible to the same polyhedrosis virus, *Borrelina campeoles* Steinhaus. According to the present concept, intergeneric susceptibility to the same virus is regarded as rather unusual since insect viruses in general are assumed to be highly species-specific. However, the concept of host specificity of insect viruses may require significant alteration because of the increased number of reports on the transmission of insect viruses from one host species to another.

Bergold (1943) cited several early workers who had reported the transmission of virus disease from one insect species to another. Steinhaus (1949), however, stated that the early claims of interspecies susceptibility of silkworm jaundice with other Lepidoptera and even with a beetle were highly questionable and should be retested. The interspecies susceptibility to virus diseases among several lepidopterous species was reported by Bergold (1943). He found that the larvae of the gypsy moth (*Porthetria dispar* (L.)), the nun moth (*Lymantria monacha* L.), and the pine moth (*Dendrolimus pini* L.) were partially susceptible in the laboratory to *Borrelina bombycis* Paillot, which caused jaundice of the silkworm. He was able to transmit to some silkworm larvae the polyhedrosis virus, *Borrelina reprimens* Holmes, of the gypsy moth. The larvae of the gypsy moth and the pine moth were partially susceptible to the polyhedrosis virus, *Borrelina efficiens* Holmes, of the nun moth. In a serological study, Bergold found that the virus of the nun moth was more closely related to the virus of the gypsy moth than to that of the silkworm. Jucci (1944) implied that the larvae of *Philosamia ricini* (Boisd.) and *P. cynthia* (Drury) were susceptible to the polyhedrosis virus of silkworm. Steinhaus (1952) found that the polyhedrosis of the South American species, *Colias lesbia* (Fabricius), had polyhedra and virus particles that were essentially similar to those from the polyhedrosis of the North American species, *Colias philodice eurytheme*. He was able to transmit this virus disease readily to the North American species. This transmission indicated that the South American virus might be the same as *Borrelina campeoles* of *Colias philodice eurytheme*. Recently Smith and Zeros (1952) succeeded in transmitting polyhedrosis viruses not only between closely related insect species (*Tineola biselliella* Hummel and *Tinea pellionella* L.) but also to unrelated species. They were able to infect the larvae of the small tortoise shell butterfly, *Aglais urticae* L., and the peacock butterfly, *Vanessa io* (L.), with

viruses obtained from the larvae of the following insects: the currant moth, *Abraxas grossulariata* L.; the scarlet tiger, *Panaxia dominula* (L.); the cream-spot tiger, *Arctia villica* L.; and an American species of silk moth, *Telea polyphemus* Cramer. They also infected the larvae of the privet hawk moth, *Sphinx ligustri* (L.), with the viruses found in the scarlet tiger, currant moth, and the silk moth. The larvae of the cinnabar moth, *Callimorpha jacobaeae* L., were found susceptible to the viruses of the scarlet tiger, the muslin moth (*Cycnia mendica* Clerck), the privet hawk moth, and the silk moth.

The identity of the viruses of *Pieris rapae* and *Colias philodice eurytheme* is supported by the following evidence: (1) the two viruses are mutually cross-transmissible to both insect species; (2) each virus remains infectious to its original host after it has been transmitted to the other host; (3) the symptoms of polyhedrosis are characteristic of the host species regardless of whether the host has been infected with the virus of the other species or with its own virus taken from the other species; (4) the size and shape of the polyhedra and virus particles of the two diseases are essentially the same; (5) the histopathology of both diseases appears similar; (6) serological tests have indicated close relationship between the two viruses; (7) attempts to activate latent virus infection in the imported cabbageworm have failed.

The size and shape of the polyhedra obtained from infected *Pieris rapae* are approximately the same as those given for the polyhedra of *Colias philodice eurytheme* by Steinhaus (1948). There is a slight difference in the widths of the two viruses, but their lengths are nearly identical. Steinhaus (1948) gave the measurement of the virus particles from *Colias philodice eurytheme* as approximately 40 by 300 millimicrons. In *Pieris rapae*, most of the particles are 51 to 60 millimicrons by 281 to 300 millimicrons. The slight difference in the widths of the virus particles of *Pieris rapae* and *Colias philodice eurytheme* may have been caused by the different methods of preparing the virus particles for electron microscopy, or perhaps the virus particles of *Pieris rapae* were still enclosed in a membrane, while those of *Colias philodice eurytheme* were free. The polyhedra and virus particles from both insect species are compared in fig. 1.

In the polyhedrosis of *Pieris rapae*, each polyhedron contains approximately 3 to 19 virus bundles, with an average of 9 virus bundles. Since some of the virus bundles may have passed out of the envelope during the process of dissolution, there may be more virus bundles per polyhedron. Hughes (1950) has also observed only a small number of virus bundles per polyhedron in the polyhedrosis of *Colias philodice eurytheme*. Under the electron microscope, he has found three or four virus bundles per polyhedron, but under the dark field microscope, he found eight or ten bundles in a polyhedron.

The histopathology of the polyhedrosis in the imported cabbageworm is similar to that of the disease in the alfalfa caterpillar. In both hosts, the virus infects mainly the fat, hypodermis, and tracheal matrix.

The serological tests indicate the identity of or a close relationship between the viruses from the two host species. The polyhedra of both hosts agglutinated to high titres with either the homologous

antiserum or that of the other virus. The high titres differentiated these polyhedra from those of other insect species which had given positive agglutination at a low titre. In the precipitation test, only the fraction of the polyhedra that was soluble in alkali gave a positive test. The insoluble fraction which included the virus particles did not give a positive reaction.

The cross transmission of the polyhedrosis between the alfalfa caterpillar and the imported cabbageworm appears to be an infection resulting directly from the virus inoculum rather than the activation of a latent virus, because no virus disease developed in *Pieris rapae* when healthy larvae were fed either the polyhedra of other virus diseases or the body contents of a healthy larva of *Colias philodice eurytheme*. Neither did a virus disease develop in *Colias philodice eurytheme* when the body contents of a healthy larva of *Pieris rapae* were fed to its larvae. Moreover, the attempts to produce a virus disease in the imported cabbageworm by cold treatment, by heat treatment, and by feeding the larva with potassium nitrite or hydroxylamine have not succeeded.

The polyhedrosis in *Pieris rapae* and that in *Colias philodice eurytheme* differ from each other in the symptoms and the susceptibility expressed by the two host insects. *Colias philodice eurytheme* is highly susceptible to polyhedrosis (Steinhaus, 1948; Steinhaus and Thompson, 1949; Thompson and Steinhaus, 1950), but *Pieris rapae* is fairly resistant to the disease. Out of 124 cabbageworms which were fed the polyhedrosis virus, 80 or 64.5% of them died with polyhedrosis. In addition, the period of infection is much longer in *Pieris rapae*, 11 to 21 days, than in *Colias philodice eurytheme*, 5 to 10 days. The infection of polyhedrosis in *Colias philodice eurytheme* proceeds rapidly to a complete wilt of the larva, while in *Pieris rapae* the infection progresses very slowly and, although the integument becomes fragile, generally the larva does not assume the characteristic wilted condition. These differences in the reaction of two unlike hosts are to be expected, especially if the hosts are as distantly related phylogenetically as are *Pieris rapae* and *Colias philodice eurytheme*.

SUMMARY

A polyhedrosis virus has been found in the imported cabbageworm, *Pieris rapae* (L.), reared in the laboratory. This is the first record of a polyhedrosis in *P. rapae*. The disease is infectious but not highly virulent to the cabbageworm, which dies in 11 to 21 days.

Most of the polyhedra vary from 0.7 to 2.7 microns in diameter, with an average of about 1.4 microns. The virus particles range from 41 to 90 millimicrons wide and 151 to 340 millimicrons long, but most of them are 51 to 60 millimicrons by 281 to 300 millimicrons.

The polyhedrosis virus of *Pieris rapae* appears to be identical with *Borrelina campeoles* Steinhaus of *Colias philodice eurytheme*. The following evidence supports the intergeneric susceptibility to the same virus: (1) the viruses of both insect species are transmissible to either host insect; (2) each virus remains infective to the original host after passage through the other host; (3) the size and shape of the polyhedra and virus particles of the two viruses are essentially the same; (4) each

host exhibits its own pathologic symptoms when infected with either virus; (5) the histopathology within the two host species is similar; (6) serological tests indicate a close relationship between the two viruses; (7) treating the imported cabbageworm with high temperature or subfreezing temperature or feeding them potassium nitrite, hydroxylamine, and foreign proteins failed to activate any latent form of virus in the larvae.

The difference between the two polyhedroses lies in the pathologic symptoms and susceptibility expressed by the two host species.

The polyhedroses of several insect species other than that of *Colias philodice eurytheme* are not infectious to *Pieris rapae*. Attempts to transmit the polyhedrosis of *Pieris rapae* to several insect species other than *Colias philodice eurytheme* also failed.

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ENTOMOLOGY (MEDICAL AND VETERINARY) INCLUDING INSECTICIDES AND INSECT AND RAT CONTROL, by D. N. Roy and A. W. A. Brown. Pp. ix + 413, 193 figs., 6 pl. Excelsior Press, 11A, Hidaram Banerjee Lane, Calcutta 12, India. 1954. Price, $8.00.

The first edition of this book was written during World War II, at a time when scientific intercommunication between nations was difficult, and published in 1946. The present volume, labelled a second edition, is a revision of that work, with the addition of a section on insecticides and insect control written by Dr. Brown, (whose "Insect Control by Chemicals" was reviewed in the ANNALS, vol. 45, p. 422, 1952). The book, therefore, consists of two distinct and complementary parts which differ in their treatment of the subject and which require separate evaluation.

The first part, "Entomology (Medical and Veterinary)" (pp. 1–290) deals with the life histories, bionomics, and identification of medically important arthropods. The emphasis, naturally, is on the medical entomology of India, though the author attempts to consider the subject as it applies to other parts of the world as well. However, he shows a lack of familiarity with recent (North and South) American Literature, even in respect to those aspects which deal with subjects of importance in his own country. Life cycles and the biology of pathogenic organisms transmitted by insects are discussed much more briefly than is usual in a text of medical entomology, or are disregarded altogether, the student apparently being expected to get such information from texts in parasitology. On the other hand, there is much information dealing with methods and techniques, a feature which will increase the reference value of the book. Unfortunately, the text is marred by numerous errors, omissions, and inaccuracies, and in some passages the meaning becomes lost or confused.

The second part, "Insecticides and Insect and Rat Control" (pp. 291–406) forms a section which will be very useful for reference purposes. The treatment seems to be as reasonably up-to-date as could be expected in this fast-changing field. The five chapters are entitled "The chemistry of insecticides", "The toxicology of insecticides", "The application of insecticides", "Insecticidal control of noxious Diptera", and "Insecticidal control of other species of medical importance". Repellents are considered along with insecticides, in the proper context. The development of resistance in medically important arthropods, the genetics and physiology of resistance, and its significance in relation to control practices, are discussed. The part closes with a consideration of rat control, a topic not foreign to the general subject, in consideration of the importance of reservoir control in relation to vector control, but one which usually does not find a place in textbooks of medical entomology.—M.T.J.

A STUDY OF THE BIOLOGY AND LIFE HISTORY OF PROSEVANIA PUNCTATA (BRULLE) WITH NOTES ON ADDITIONAL SPECIES (HYMENOPTERA: EVANIIDAE)

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The Evaniidae are among the least known and most unusual of the parasitic Hymenoptera. The purpose of this study is to summarize the known information about these parasites and to extend this knowledge by investigations on their biology and life history.

Studies were conducted in Ohio from 1950 through 1952 and a number of observations were made on the hosts of these parasites as well as upon the parasites themselves. Specimens collected were identified by the writer, and then sent to Henry K. Townes or Luella M. Walkley for verification. The descriptions of species and the names used by the writer are based upon the work of Townes (1949).

At the present time there are four genera and eleven species of Evaniidae described from the Nearctic region. Nine of these species are native, and two have been introduced.

Evania appendigaster (Linn.), one of the introduced species, was probably of oriental origin but now occurs in most of the tropical and subtropical parts of the world. In the United States it is common in Arizona and in the cities of the Gulf and Atlantic States as far north as New York City. The other introduced species, *Prosevania punctata* (Brullé) (Figs. 1 and 2) is a native of the Mediterranean Region and has been established from New York and Ohio south to Georgia. During the course of this study *Prosevania punctata* was found abundantly throughout Ohio, while *Evania appendigaster* was not found in this area.

The distribution of our native species of evaniids is not well known. *Hyptia harpyoides* Bradley has a more northern range than any other evaniid in the Nearctic region. It occurs in eastern United States, and as far west as Minnesota and Kansas. *Hyptia thoracica* (Blanchard) occurs from the Atlantic States west to Michigan and Kansas. *Hyptia thoracica* and *Hyptia harpyoides* are the only evaniids recorded from Canada. *Hyptia reticulata* (Say) ranges from the Atlantic States west to Michigan and Kansas; it has been collected in southern Mexico. *Hyptia femorata* Townes at present is known only from Texas, while *Hyptia floridana* Ashmead has been collected in Florida, Georgia, and Louisiana. *Hyptia oblonga* Townes has been collected in Arizona, Alabama, Georgia, and Mexico.

The genus *Evaniella* is represented in the Nearctic region by three species, one widespread in eastern United States and the other two

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restricted in distribution to New Mexico and California. *Evaniella semaeoda* Bradley occurs from the Atlantic west to Michigan and Kansas. Along the Atlantic coast it has been collected as far north as Rhode Island, and in the central states as far west as Livingston County, Michigan. *Evaniella neomexicana* (Ashmead) has been recorded from New Mexico, and *Evaniella californica* (Ashmead) from California.

The Evaniidae are parasites of roach oothecae. Records of these wasps having been reared from oothecae are mentioned by Ashmead (1901), Fahringer (1922), Rau (1940), Cros (1942), Townes (1949), and Edmunds (1952a and b). Three species of native evaniids *Hyptia harpyoides*, *Hyptia reticulata*, and *Hyptia thoracica*, are known to parasitize wood roaches. *Hyptia harpyoides* has been recorded as a parasite of *Parcoblatta virginica* (Brunner), and *Hyptia reticulata* and *Hyptia thoracica* as parasites of *Parcoblatta pensylvanica* (De Geer).

The two introduced species of evaniids, *Evania appendigaster* and *Prosevania punctata*, are commonly found in buildings, and are parasites of domestic roaches. *Evania appendigaster* has been recorded as a parasite of the Oriental roach *Blatta orientalis* Linn., the American roach *Periplaneta americana* (Linn.), and the Australian roach *Periplaneta australasiae* (Fabricus). *Prosevania punctata* also parasitizes the Oriental and American roaches, and in addition the German roach *Blattella germanica* (Linn.).

Some evaniid species are not common and their hosts are unknown; this is true for the three species of *Evaniella* and for *Hyptia femorata*, *Hyptia floridana* and *Hyptia oblonga*.

METHODS

Field Studies. Evaniids may be collected in the field as adults or be reared from parasitized oothecae. A knowledge of cockroach habits is necessary if one is to obtain and study their parasites. Investigations on the biology of roaches were made along with studies on the evaniids, to aid in an understanding of the parasites.

Domestic roaches often deposit their egg capsules in areas of buildings which are difficult to reach. The Evaniidae, because of their small size, can enter these areas to parasitize the capsules, but a person cannot collect there. It was found easier and more successful to collect adults of *Prosevania punctata*, than it was to try to find parasitized egg capsules in buildings.

A different technique was necessary for the evaniid parasites of the wood roaches. Specimens of *Hyptia* were seldom seen or collected in the adult stage, but they could be readily obtained by collecting wood roach egg capsules and keeping them in closed containers until the parasites emerged. Parasitized egg capsules were collected in the field, generally from under the loose bark of dead trees. From these capsules were reared out a variety of parasites, including *Hyptia thoracica* and *Hyptia harpyoides*. Methods used for collecting wood roach capsules, together with notes on wood roach habits and parasites, are described in a previous paper, Edmunds (1952b).

After the egg capsules were collected in the field they were candled by holding them in front of a bright light. The light will pass completely through an empty capsule, or show the outlines of an evaniid

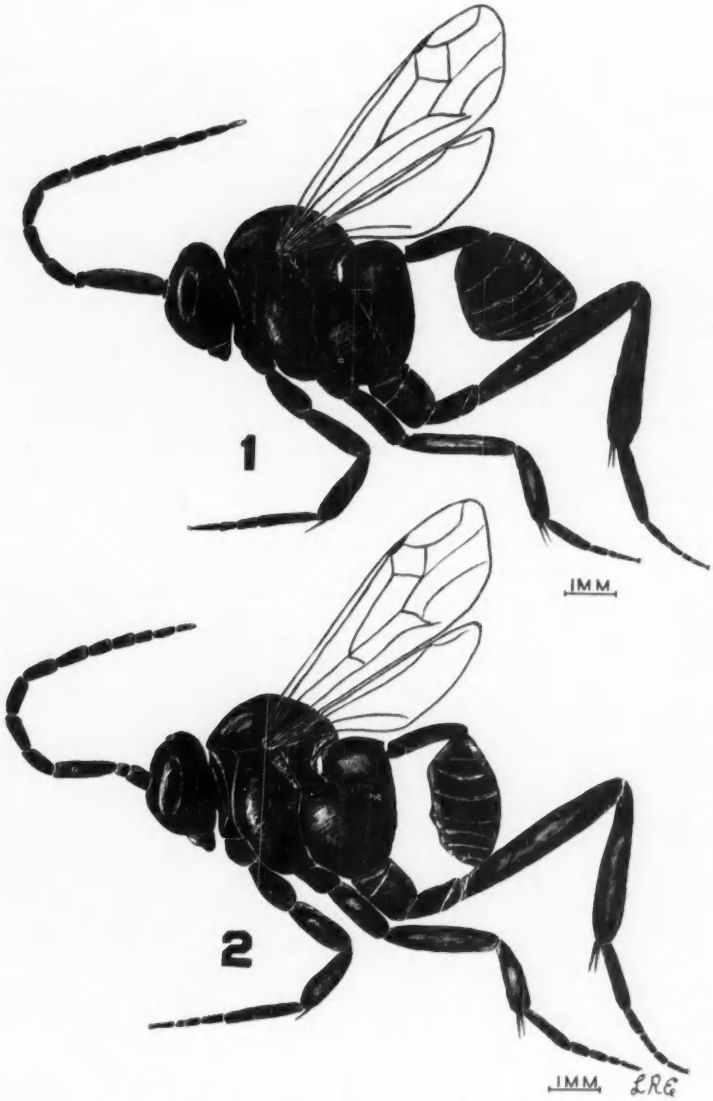


FIG. 1. *Prosevania punctata*. Lateral view of adult female.
FIG. 2. *Prosevania punctata*. Lateral view of adult male.

larva if one is present in the capsule. This method was used on several hundred egg capsules which were collected over a period of two years, and was found to be very successful.

Laboratory Methods. Cultures of *Prosevania punctata* were started from adults collected in the vicinity of Columbus, Ohio. Several dozen adult wasps were captured during a period of about two years. These wasps were placed in a glass-topped rearing cage, and different kinds of roach capsules were then placed on the floor inside of the cage. Oviposition was observed to take place many times under these conditions.

The egg capsules were removed daily and a fresh supply was put in their place. The capsules which were removed from the cage were placed in small glass jars, the mouths of which were covered with fine white organdy held in place by rubber bands. The bottles were then placed in an outdoor insectary and left until the parasites emerged.

The natural food of the evaniids was unknown to the writer, so a number of different kinds of flowers were placed in the cage with adults. These flowers were put into shell vials which were filled with water. Cotton was packed around the stem at the mouth of the vial to prevent the wasps from crawling down into the vial and drowning. Observations were then made as to the types of flowers the wasps visited. Pollen grains were observed on adults of *Prosevania punctata* but their source was not determined.

Methods of Studying the Immature Stages of Evaniidae. In order to obtain a series of immature stages, the ovipositing female *Prosevania punctata* were observed closely when roach capsules were placed inside the oviposition cage.

Oothecae were removed from the cage after parasite had oviposited into them and placed in separate vials. Each vial was marked with the date of parasite oviposition. The capsules were then incubated and dissected at definite intervals of time, and the larvae were removed for study.

Since dissection was necessary for the study of the internal phase of the parasite's life, the following method was developed. The egg capsule was held between the thumb and forefinger, and the ridge along one edge of the capsule was cut away with a very fine scissors. A needle was inserted between the edges of the capsule and the edges were gradually spread apart. The contents of the capsule were emptied into a watch glass containing an 0.85 percent sodium chloride solution. The parasites could then be sorted out of the debris and would remain alive in this solution for some time, and observations of living material could thus be made. After observing the larvae in this salt solution, they were transferred to K.A.A.D.[2] for a short time, usually less than an hour; after being killed in this solution they were preserved in 80 percent alcohol.

[2]K.A.A.D. consists of 1 part kerosene, 10 parts of 95% ethyl alcohol, 2 parts acetic acid, and 1 part dioxan. See Peterson (1949).

Biology of the Evaniidae

The Adult

Emergence from the Ootheca. The evaniid wasp working from the inside of the egg capsule pierces the hard cover with both mandibles. The shiny black mandibles puncture two holes in the wall, and are then closed by the wasp so that the tips meet outside of the egg sac wall, and by an inward pull the wasp breaks a small hole. After making this initial opening, the parasite begins to bite small pieces from around the edge of the opening, thus gradually enlarging it (Fig. 3). As the hole is enlarged, the head and front parts of the thorax becomes visible to the observer.

The wasp can be seen moving and stretching as it gradually enlarges the aperture and may make an attempt to emerge through the hole by pushing with its head. If the opening is too small or not the proper shape, the wasp will pull back into the capsule and begin to cut again. Systematic cutting at the edge of the opening is carried on until the hole is large enough for the passage of the wasp's body. A wasp may make several attempts at emergence before it succeeds. Finally the front legs are forced through the hole, and the wasp uses these to pull the rest of the body through. Upon emergence from the ootheca, the wings of the adult are fully expanded, the body appears quite as dark and dry as those of older adults.

The emergence opening is made at the top side and close to one end. An examination of thirty capsules of *Hyptia thoracica* shows that the same number of adults emerge from one end of the egg capsule as emerge from the other. In the genus *Hyptia*, the emergence hole is about 2 mm. in diameter, but the size varies a great deal from specimen to specimen. In *Prosevania punctata*, a larger wasp, the emergence hole is about 3 mm. in diameter.

Mating Behavior. *Prosevania punctata* adults walk about continuously inside any confined area, and whenever a female passes near a male, the male follows behind the female and vibrates his antennae rapidly, while his entire body quivers and shakes. Sometimes two males will show similar excitation over the same female. If the female stops to drink or rest, very often a male will approach her and touch her with his antennae. The usual reaction of the female is to walk or move rapidly away. The male will then run about for a few seconds as though seeking her.

Copulation was observed taking place in a cage containing 12 *Hyptia thoracica*. A male and female with the abdomens locked together fell from the side of the cage to the floor. Upon landing they still remained attached together for a few seconds. Upon breaking apart the female ran up the side of the cage while the male remained motionless on the floor of the cage. This act of copulation was extremely rapid, lasting only a few seconds.

Oviposition. Oviposition by *Prosevania punctata* was first described by Edmunds (1952a), who observed oviposition in egg capsules of the American roach, *Periplaneta americana*. Since that time this evaniid has been found to parasitize egg capsules of the Oriental roach,

Blatta orientalis. Haber (1920) gave a description of *Evania appendigaster* ovipositing into egg capsules of the Oriental roach.

Live female American roaches carrying egg capsules were placed in the cages with the wasps. Some of the female wasps paid a great deal of attention to the egg capsules being carried by the roaches, but oviposition was never observed except in egg capsules which had been dropped by the roach. Very often the American roaches glue their egg capsules into a corner of the cage. These capsules received more attention from the wasps than the cleaner ones which were merely dropped.

When several egg capsules were exposed at the same time in a cage, the reaction of the female wasp was to walk back and forth over the capsules many times. She would push them around with some vigor, turning some of the capsules over and seemingly examining them closely. Many times she would walk away after the first examination, and come back in a short time to examine the same oothecae again. This might go on for some time before oviposition would occur, and many times oviposition would not take place at all.

In the actual act of oviposition the female approaches the egg capsule and examines it very carefully by stroking it many times with rapidly vibrating antennae. Finally she settles down, usually on her side, laying on the cage floor with her ventral surface facing the long axis of the egg capsule. While in this position the ovipositor is extended and punctures the egg capsule. The usual position of the wasp is to face the flanged edge of the egg capsule, but oviposition has been observed taking place from the opposite side of the ootheca, and in one instance directly down into the side of a capsule. It is assumed that although there is a preferred position for oviposition, the position is not an obligatory one. Considerable variation was noted in the length of time spent by *Prosevania punctata* in ovipositing. Ten ovipositions were timed and ranged from 16 to 62 minutes with a mean average of 29 minutes.

In an egg capsule opened a few days after evaniid oviposition was observed an evaniid larva was found, not a newly hatched form. This observation indicates that the adult wasps cannot always detect capsules which have been previously parasitized. The last instar larva found within this capsule appeared normal, and it is assumed that the second egg was eaten by the old larva.

On three different occasions it was noted that within a few hours after evaniid oviposition had taken place the nymphal roaches emerged from these parasitized oothecae. These observations suggest that parasite oviposition was too late for successful egg development. Oviposition by the wasp must take place before the young roaches are in their last stages of pre-emergence development if a parasite is to be produced.

Feeding Habits of Adults. Townes (1949) states that five specimens of *Hyptia floridana* were collected near Miami, Florida, while feeding on the petiolar nodules of *Ricinus communis*. Crosskey (1951) states that adults of *Evania appendigaster* emerge in summer, and may be found visiting such flowers as *Petroselium crispum* (Mill.) and *Foeniculum vulgare* Mill.

FIG. 3. Photograph of adult *Hyptia thoracica* (Blanchard) chewing an emergence hole through the wall of an egg capsule of the wood roach, *Parcoblatta pensylvanica* (DeGeer). From a kodachrome by D. J. Borror and L. R. Edmunds.

Robertson (1928) listed the visits of various kinds of insects to a number of species of flowers. His observations show that *Hyptia reticulata* visited parsnip, *Pastinaca sativa*. This plant is in the family Umbelliferae, a group known to be attractive to many of the Ichneumonidae.

Blooms of *Asmorrhiza longistylis*, a closely related plant, were placed in the cage with a dozen specimens of adult *Hyptia thoracica*. The insects were attracted to the flowers and fed on them.

Adult *Prosevania punctata* in captivity were fed 5 percent honey water. They would live on this type of food up to 20 days after being captured. The wasps become less active as they grow older and remain close by the container of honey water to feed.

Parthenogenetic Reproduction. Cros (1942) observed ovipositions by an unfertilized *Prosevania punctata* female but no larvae developed from the unfertilized eggs. Ovipositions by an unfertilized female *Prosevania punctata* and the subsequent emergence of new adults as a result of these ovipositions have been observed by the writer.

In October, 1950, a lone female wasp was collected from a window in the basement of a home in Columbus, Ohio. In captivity she oviposited into egg capsules of the American roach, and produced three wasps all of which were males. This observation suggested that parthenogenetic reproduction might occur in the Evaniidae, and was worthy of further study.

In July, 1951, another adult female was captured in the Botany and Zoology Building on The Ohio State University campus. This wasp was given access to a number of Oriental and American roach capsules. Oviposition was observed, and the offspring of this female were four males and three females which were removed from the cage as they emerged. The last of the offspring to emerge was a female, which came from a capsule of *Blatta orientalis* on January 9, 1952. This female was placed in a cage, and egg capsules were exposed to her in hopes of obtaining evidence of parthenogenetic reproduction. At the time she emerged there were no other adult wasps in the writer's cultures. The wasp emerged inside a small glass jar, over the mouth of which was stretched a covering of fine white organdy. The wasp on emergence was moved into a glass and organdy cage in which all of the cracks had previously been sealed with wax. These circumstances preclude any possibility that the female could have been fertilized. She began to oviposit within from three to four hours after emerging from the egg capsule.

The egg capsules in which the wasp oviposited were taken from the cage each evening and placed in glass jars which were labeled with the date of parasitization. One hundred and twenty-three capsules were exposed to this wasp for parasitization. These consisted of 101 Oriental capsules, and 22 American capsules. Fresh oothecae were collected from the roach cultures and placed in the cage daily.

On February 7, 1952, one of the egg capsules was dissected and found to contain a pupa in an early stage of development. This pupa was twenty-six days old and was pearly white in color except for very light pigmentation in the eyes, and the abdomen showed no segmentation. On February 28, 1952, 45 days after the virgin female had

oviposited, her first progeny emerged from an Oriental roach capsule. Only three parasites emerged, the last eight days later. These three together with the pupa previously dissected out by the writer, made a total of four successes. The adult wasps were all males.

Prosevania punctata reproduces sexually under normal conditions, but is capable of parthenogenetic reproduction. In the example given above, the parthenogenesis was natural (spontaneous), since it was not induced by any artificial stimulus, and complete, because the offspring reached maturity. Since only males were produced by the unmated female, the Evaniidae have arrhenotokous parthenogenesis.

Overwintering and Group Emergence. The *Hyptia* attacking the egg capsules of our native species of roaches overwinter inside of the egg capsule, after first consuming the contents. *Hyptia thoracica* was found overwintering inside of the ootheca of *Parcoblatta pensylvanica* and *Hyptia harpyoides* in those of *Parcoblatta virginica*. The overwintering stage of *Hyptia* was found to be the last instar larva. In the late spring of 1951, 31 *Hyptia* were observed emerging from egg capsules collected in the woods. The emergence occurred from June 10th to June 20th.

Collections of egg capsules in the woods were made in such a way that the oothecae found in one particular place were kept together for study. The *Hyptia* which emerged from these capsules did so in groups, emerging almost simultaneously from those capsules collected in one niche and at a different time than those from capsules collected in a different niche. All of the wasps in the different collections emerged within a period of ten days.

Oothecae collected in a localized area may include several which are parasitized, and the wasps in the capsules all emerge at about the same time. The emergence records show that the egg capsules are parasitized in groups. This may be due to the habits of the wood roaches which congregate in certain areas and often drop their egg capsules close together. It is possible that the adult female evaniid on coming to such a spot would parasitize more than one capsule, and in fairly rapid succession; this might account for group emergence, but such female behavior was not observed.

Prosevania punctata overwinter as larvae or pupae in egg capsules of domestic roaches, and in the vicinity of Columbus, Ohio, emerge in the last two weeks of May and the first week of June. Under laboratory conditions a generation of *Prosevania punctata* emerged in January. There is no evidence that such a generation occurs under natural conditions, as there are no records in the museums at Columbus, Ohio, of *Prosevania punctata* adults being collected between October and May.

General Adult Behavior. The adult wasps continually use the hind legs to rub or clean the sides of their bodies. The wasps stop, balance themselves on the first two pairs of legs and raise the hind pair up over the back. The hind legs are then rubbed backward along the sides of the body. Occasionally they raise the hind legs in the air and rub them together.

In collecting adult *Prosevania punctata* it was observed that the usual means of locomotion of the wasps is to walk, and if disturbed, they fly only a short distance and resume walking. This trait was

noted also in caged specimens. They continually walk about inside their enclosures, seldom flying. They use their antennae to touch the floor in front of them as they move about, examining any objects they encounter.

In buildings where there is an abundance of roach capsules these parasites probably reproduce for many generations without ever leaving the premises. Such an occurrence was reported in a home in Worthington, Ohio by Edmunds (1953). In this case the people were greatly annoyed by the regular, periodic appearance in their living quarters of a number of *Prosevania punctata* adults.

The Immature Stages

The only stage of the evaniid life cycle that is not spent inside of the roach egg capsule is the short period of adulthood. The length of time from oviposition to the emergence of the adult varies from 45 to 177 days in *Prosevania punctata* and periods of a year in the genus *Hyptia*, which seems to have only a single generation.

It was necessary to dissect the roach capsules to study the immature stages of the evaniids, and this dissection resulted in the death of the parasite. Descriptions and drawings of the immature stages were made from preserved specimens.

The Egg. In *Prosevania punctata* a single egg is deposited in the roach egg capsule. The female wasp inserts her ovipositor through the hard outer covering of the roach ootheca after it is dropped. The parasite egg has been found by the writer between the roach eggs, rather than inserted inside a roach egg. The position of the evaniid egg in the roach egg capsule depends on the position assumed by the wasp in ovipositing, and it varies from capsule to capsule.

The oviposition pattern of *Prosevania* differs from that of *Zeuxevania splendidula* Costa, which develops in the egg capsules of *Loboptera decepiens* Germ. in Europe. Genieys (1924) described *splendidula* as depositing its eggs[4] in the capsule of the roach before the cover is completely hardened.

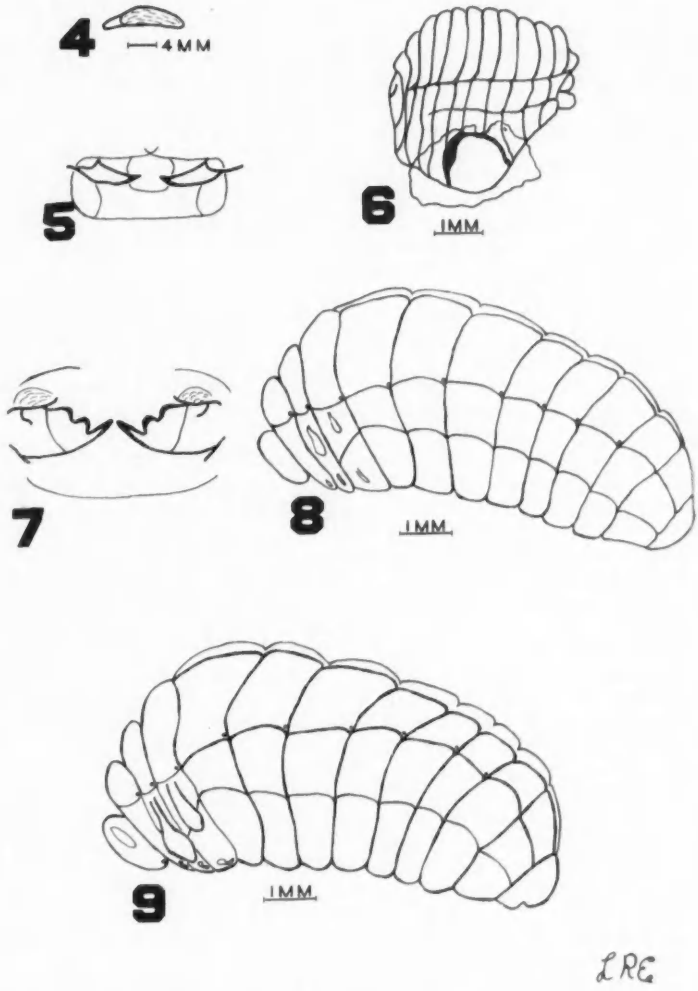
The egg of *Prosevania punctata* is rather spindle-shaped in outline and is slightly curved (Fig. 4). Both ends of the egg are smoothly rounded, and the chorion or outer covering is smooth. The egg is grayish-white, with the narrow end lighter in color. Clausen (1940) applies the name "hymenopteriform" to this shape of egg.

The *Prosevania* egg is about 1.26 mm. in length and 0.25 mm. in width. It appears to be very large to have passed through the slender ovipositor and this size relation might account for the length of time required for oviposition.

Larval Stages

First Larval Instar. The first larval instar of *Prosevania* (Fig. 6), two weeks after oviposition has occurred, is small and almost spherical in shape. It is 3 mm. in length and about the same width, and it appears like a small white ball. There are two lateral ridges on the

[4]Genieys (1924) found cockroach oothecae having as many as four oviposition scars but these egg cases never contained more than two parasite eggs.



FIGS. 4–9. *Prosevania punctata*. 4, egg; 5, mouthparts of first stage larva; 6, lateral view of first stage larva two weeks after oviposition; 7, mouthparts of third stage larva; 8, lateral view of second stage larva; 9, lateral view of third stage larva.

larva which project out and can be seen easily. On the ventral surface of the first stage larva is a small slightly projecting area around the edge of which is a ring, a little darker in color than the white body of the larva.

The first larval instar has a smooth integument with no visible spines or setae. Segmentation is difficult to determine on the compact ball-like larva, but is distinct enough for one to make out 12 body segments (including the one with mouth parts). The body is white in color. A pair of light brown mandibles occur on the first segment. They are opposable and are of a simple one-toothed type (Fig. 5). There are a few small brown patches around the mouth parts. The definitive head is not evident at this stage of development. The digestive, nervous, and tracheal systems appear whitish in color, and are not clear through the body integument.

Development after the first instar tends toward a typical "hymenopteriform" type. The principal change to the hymenopteriform type seems to occur in the second instar in *Prosevania punctata*.

Second Larval Instar. After the first larval stage is passed the development of the parasite is very rapid. The second larval instar, in comparison with the first has become elongated and grayish-white in color. There are twelve indistinct body segments and a head (Fig. 8). White and indistinct tracheal openings are present. The head is well differentiated but lacks any sign of eyes. The wing buds and leg buds are evident but are small and difficult to distinguish. The mandibles of the second instar are similar in appearance and shape to those of the third instar but are smaller.

Third Larval Instar. The third instar is dull creamy white in color. The body is thickest near the mid-region, and is attenuated somewhat at the ends. The head is well developed and bears dark brown sclerotized mandibles which have three denticles (Fig. 7). There appear to be twelve segments and a head. Each body segment is extended dorsally to form a projection, giving the effect of a series of humps on the back when the larva is viewed laterally. A groove extends down the sides of the body from head to tail, making the larval body appear to be divided into plates (Fig. 9).

The tracheal system of white tubes can be partially seen through the integument. In some of the larvae a system of indistinct white tracheal openings can be noted between the segments. These tracheal openings are found between each pair of body segments from the first to the tenth. No tracheal openings were observed between the 10th and the 11th or the 11th and 12th segments. As far as could be determined the larvae of *Prosevania punctata* have nine tracheal openings on each side of the body.

In this stage of development the wings are evident as saclike outpouchings of the body, and the legs are discernable as small projections on the ventral side of the first three body segments. The eyes are present but lack pigment.

The larva is completely covered by a clear transparent membrane. This is probably the outer cover which has been distended because of some effect of the killing solution. This membrane is firmly attached around the head region and was very difficult to remove without destroying the specimen.

Several last stage larvae were found to vary from 8.5 to 10.0 mm. in length and from 3.8 to 4.5 mm. in width.

Pupal Stages

Upon dissecting the egg capsule the pupa was observed in all instances resting upon a spongy, homogeneous, gray mass which filled the curved portion of the egg capsule opposite the ridge. This substance was soft, easily broken up, and probably consisted of an accumulation of waste products from the larval stages.

The pupa of *Prosevania punctata* was found to be of an exarate type, with the wings and legs free from any secondary attachment to the body. The pupa when removed from the egg capsule was found to be covered with a brown membrane which could be removed easily with a needle.

Early Pupal Stage. In the early pupal stage (Fig. 10) the evaniid is pure white in color except for the eyes; the eyes begin to darken almost immediately, turning red and then black. The wings, legs, and antennae are distinct and well formed. The most distinguishing feature of the early pupal stage is that the abdomen is connected to the thorax by a broad base; there is little or no evidence of the narrow constriction evident in later pupal and adult stages. The abdomen shows no evidence of external segmentation. The mouth parts are evident and well defined but lack pigment. On the underside of the caudal end of the abdomen the external genitalia appear as small white rod-like extensions. The ocelli are indistinct.

Intermediate Pupal Stage. As the pupa develops (Fig. 11) there is an increase of pigmentation in some parts of the body. The head and thorax turn black, the petiole dark brown, while the abdomen remains white. The legs become dark brown at the base and lighter distally. The antennae remain white. The mouth parts become pigmented and the mandibles distinct. At this stage of development the abdomen is connected to the thorax by the narrow constricted petiole characteristic of the adult. The abdomen is still round and is just beginning to show the development of plates and segmentation. The ocelli are distinct and are reddish-brown in color.

Late Pupal Stage. In the late pupal stage (Fig. 12) the pigmentation of the body is nearly completed. The last portions of the body to turn black are the antennae, the middle region of the abdomen, and the tips of the legs. The thorax and petiole become black before the abdomen begins to darken. In this late pupal stage there are two rows of dark plates forming on the abdomen. Seven of these plates appear dorsally and seven ventrally, indicating that the abdomen is seven segmented. The segmentation of the abdomen becomes difficult to see after fusion has occurred. The two rows of plates are separated by a white unsclerotized area as seen in Fig. 12. The ocelli have become black. The external genitalia appear as a group of small projections on the under side of the abdomen. The abdomen is still round but as the sclerites develop the abdomen begins to compress laterally and gradually the white area is covered and enclosed.

The principal external changes that occur in the pupa as it increases in age are: progressive darkening and sclerotization, and distinct segmentation.

Seasonal Abundance

The first adults of *Prosevania punctata* emerged in the field during the last two weeks of May and the first week of June in 1951. During this three-week period adults were collected in Columbus, Ohio, and roach capsules exposed to these parasites. Oviposition took place into many of these capsules. These oothecae were incubated in a screened insectary under conditions as natural as could be obtained, and were observed closely for any emergence.

The first adult wasp emerged from an egg capsule on July 17, 1951. Eleven wasps emerged during a period of nine days from July 17 to July 26. These capsules had been parasitized in late May or early June, so the parasites were from 50 to 60 days in developing. At approximately the same time that these wasps emerged in the insectary, several additional specimens were collected under natural conditions from buildings. No adults were collected by the writer in the period between broods.

The adult parasites which emerged in the rearing jars were placed in a cage, and again numerous egg capsules were exposed for oviposition. On September 24, an adult *Prosevania* emerged from a capsule parasitized on July 28, 59 days previously.

By checking all available collection records in the Insect Collection of the Department of Zoology and Entomology of The Ohio State University, the Ohio State Museum, and the writer's personal collection, the following data were obtained. There were no collections of *Prosevania punctata* earlier than May 12, and the latest (by the writer) was made on October 9. This leaves a period of seven months during the cold part of the year in which no adults have been collected.

It is assumed from the rearing records and the collection data that in 1951, there were three generations of *Prosevania punctata* in the vicinity of Columbus, Ohio. The first group emerged in late May and early June, the second group in mid-July, and the third group in late September and the first part of October.

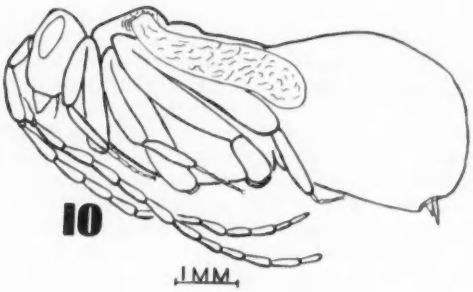
The first adult *Prosevania punctata* in the spring of 1952 was observed and collected on the window of a building on The Ohio State University campus, on June 4, nearly two weeks later than the earliest record obtained in the spring of 1951.

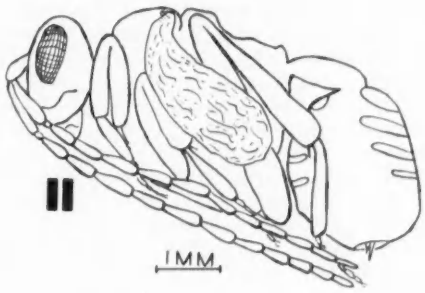
As far as could be determined, *Hyptia* has a single generation per year. *Hyptia thoracica* was found to emerge around the middle of June, from June 10 to June 20. Egg capsules of *Parcoblatta pensylvanica* collected on August 18, 1951, were candled and found to contain evaniid larvae in a fairly advanced stage of development. These larvae overwintered in the egg capsules kept in an insectary and the adults did not emerge until the spring of 1952.

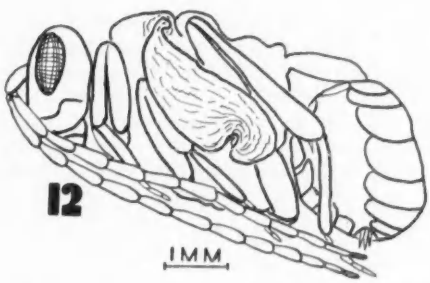
Adults of *Hyptia* are usually collected during the midsummer months in the woods, on flowers, or feeding on honey-dew. Townes (1949) states that the species of *Hyptia* appear to have a single generation a year in the north, although rearing records are scanty.

Effect of Parasitism on the Egg Capsule

Parasitized egg capsules give no gross external indication of parasitism. The only conclusive method for determining whether an egg







FIGS. 10–12, *Prosevania punctata*, lateral view of pupa. 10, early pupal stage; 11, intermediate pupal stage; 12, late pupal stage.

capsule is parasitized or not is by dissection. This is particularly true in the younger stages of the parasite before it has consumed much of the contents of the capsule.

Examinations made of egg capsules after the adult wasps have emerged demonstrates a similarity in appearance and the result of parasitism. The entire contents of the capsule are consumed during the period of larval feeding. The evaniid larva is an egg predator, feeding on one after another of the roach eggs. The speed with which this process of feeding is carried on is dependent upon temperature and humidity conditions. The warm temperatures of mid-summer cause the most rapid development. The percent of capsules parasitized by *Prosevania punctata* was not determined; however, it was possible to determine the percentage of wood roach egg capsules which contained over-wintering *Hyptia*. During the winters of 1949–50 and 1950–51 extensive collections of egg capsules were made from under the bark of dead trees, as reported by Edmunds (1952b). These capsules were collected in Franklin and Delaware Counties, Ohio. No Evaniidae were reared from capsules collected in 1949–50; however, in the 1950–51 collection, 31 evaniids emerged from 458 capsules, giving an approximate parasitism of 6.7 percent.

A similar collection of 320 egg capsules was made by the writer during the months from August, 1951, through May, 1952. These capsules were collected in Franklin, Delaware, and Allen Counties, Ohio, under situations similar to those described above. Evaniidae emerged from 9 capsules, giving an approximate parasitism of 2.8 percent.

The evaniid larva destroys anywhere from 16 to 40 potential roaches, depending on the number of eggs in the oothecae of the species parasitized.[4] As far as is known, no attempt has been made to utilize these parasites as biological control agents for roaches.

SUMMARY

The Evaniidae are parasitic during their immature stages in the egg capsules of roaches. *Prosevania punctata* and *Evania appendigaster* parasitize the egg capsules of domestic roaches. Several species of *Hyptia* parasitize the egg capsules of wood roaches. The hosts of the genus *Evaniella* are still unknown.

The evaniid attacks the egg capsule and inserts a single egg after it has been dropped by the roach. The time required for evaniid oviposition varies from 16 to 62 minutes. The immature stages of evaniids are spent in the egg capsule, and the adult emerges by chewing a hole through the wall near one end. The only stage spent outside of the egg capsule is the adult, which in captivity was fed successfully on honey water and flowers.

Oviposition by an unfertilized female *Prosevania punctata* was noted during this study. A virgin female oviposited into roach capsules shortly after she emerged. The parthenogenesis was natural (spontaneous), and complete. Only male offspring resulted.

[4]Piquett and Fales (1952) give the following data on the number of eggs contained in the capsule of various roach species: German, 40; American, 16; Oriental, 16. There is considerable difference in numbers by various authors.

Evaniids were found to overwinter in the roach egg capsules. *Hyptia thoracica* and *Hyptia harpyoides* overwinter in oothecae of wood roaches as last stage larvae. *Prosevania punctata* overwinter as a last stage larva or pupa in the egg capsule of the American or Oriental roach.

Prosevania punctata deposits a single egg between the roach eggs inside the ootheca. This egg is spindle-shaped in outline and slightly curved, 1.26 mm. in length and 0.25 mm. in width. The first larval instar is spherical in shape, 3 mm. in length and width. The second instar is elongate, a typical hymenopterous larva with more distinct segmentation and small appendage buds. The third larval instar is from 8.5 to 10.mm. in length, and 3.8 to 4.5 mm. in width; tracheae open on the first to tenth body segments, the head is well developed, and wing and leg buds are present.

The evaniid pupa is of an exarate type. As it develops the color gradually changes from white to black, and the abdomen constricts at its base to form a narrow petiole.

Hyptia thoracica and *Hyptia harpyoides* parasitized 6.7 percent of the native wood roach capsules collected during the winter of 1950–51, and 2.8 percent of the capsules collected during the winter of 1951–52. Three other species of Hymenoptera and some Diptera were also reared from wood roach egg capsules.

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These appear to be three major contributions to the study of North American bees. Dr. Timberlake's publication on *Perdita* comes after many long years of study, collecting, and determinations in that group. In his treatment, the genus is divided into nineteen subgenera, three of which, including typical *Perdita*, apparently are to be considered in the second part of the work. Dr. Michener's study of bee larvae is a welcome piece of work: from the more practical standpoint, because it presents a key which will separate many more families of Hymenopterous larvae than was previously possible; and from the more theoretical standpoint, because it presents some interesting information showing that the evolution of bee larvae has progressed independently of that of bee adults and contradicting Dollo's "law of irreversibility". Dr. Stephen's monograph appears to be a thorough-going, well-prepared piece of work. Though he reduces the 167 previously described forms to 95 species and 16 subspecies, he describes 2 species and 2 subspecies as new. Most of the illustrations are of male genitalia.

—M.T.J.

LIGHT INTENSITY AND TEMPERATURE AS FACTORS LIMITING THE MATING OF THE ORIENTAL FRUIT FLY

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In the initial field and laboratory studies of the oriental fruit fly, *Dacus dorsalis* Hendel, in Hawaii, it was observed that this insect mated only during the twilight period when the evening temperature was above 60° F. Caged flies in the laboratory exhibited a sexual behavior comparable with that reported by Back and Pemberton (1917) for the melon fly, *Dacus cucurbitae* Coq. As twilight approached, the flies became excited and produced a characteristic high-pitched buzzing sound, followed by copulation, the flies remaining in copula for 2 to 12 hours. A series of experiments was set up to determine what factors inherent in twilight conditions are responsible for this behavior, and to evaluate the limitations imposed on the reproductive potential of this fly by cool temperatures at the time mating normally occurs.

The flies used in the experiments were reared in the laboratory at temperatures ranging from 75° to 85° F. The food for the larval stages was a medium developed by the University of California Agricultural Experiment Station. This medium contains finely blended carrots, 2 percent of dried brewer's yeast, and 0.13 percent of Butoben (*n*-butyl p-hydroxybenzoate), with 2N hydrochloric acid added to bring the pH within the range of 3.5–4.5. After their emergence from puparia in moist sand, the adult flies were provided with a diet of sugar, water, and a hydrolyzed protein. They were held in 1-cubic-foot screen cages. Under laboratory conditions these flies were observed to copulate usually from 7 to 10 days after emergence and never before the sixth day.

In the first experiments flies were exposed to a reversed diurnal light pattern. The external light variations were excluded, and incandescent lamps were manipulated so that they were at full intensity from 7 p.m. to 8 a.m. Beginning at 8 am. each day the light intensity was gradually reduced from one-half the original intensity to zero by means of a variable transformer. Observations on copulatory activity were made before, during, and following the dimming procedure. They were made at extremely low intensities or, when necessary, immediately following an abrupt intensity decrease with the aid of a flashlight.

Flies from puparia that were placed in the experimental room 5 days before they emerged were observed to copulate within 10 days after emergence, but only when the lights were dimmed. This was true even when the dimming was purposely delayed for 2 to 4 hours. Flies that were transferred to the experimental room after 15 days under a normal diurnal light rhythm were observed copulating at

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the time of dimming 2 days after they were exposed to the reversed light pattern.

When the lights were returned to their full intensity for 10 to 15 minutes, the pairs broke apart. Copulatory activity was resumed when the lights were again dimmed, although it was not possible to determine whether or not the same flies were involved. After they attained sexual maturity, the flies could always be induced to copulate by either a gradual or an abrupt reduction of the light intensity by a factor of 2. Repeated experiments with the intensity of the lamps at different levels indicated that an intensity decrease as noted above—i.e. a factor of 2—was an adequate stimulus regardless of the initial intensity.

In the next experiment the copulatory responses of flies to continuous light or dark were investigated. Observations after abrupt changes from light or dark failed to reveal any copulatory activity. Under continuous darkness copulation was observed 12 days after the flies emerged from their puparia. When flies were held under continuous, uniform fluorescent lighting at temperatures from 75 to 85° F., copulation was observed 9 days after emergence and fertile eggs were obtained 4 days later. These experiments indicated that a decreasing light intensity is not an obligatory factor in stimulating copulation.

Since field observations had failed to reveal any copulation when the evening temperature was below 60° F., an experiment was made to determine whether this low temperature would inhibit copulation under the reversed diurnal light rhythm. The flies were subjected to the normal diurnal variations of light and temperature for 15 days, or until copulation had been observed. They were then placed in the experimental room with the reversed light rhythm at a temperature of 58°. The following morning no matings were noted. The same conditions were maintained for 5 days, at the end of which the temperature was allowed to rise to 65° during the night. Copulation was observed on each of the following three mornings at this temperature. The temperature was then reduced once more to 58° during the night, with negative results the following morning.

Oriental fruit flies that were allowed to attain sexual maturity under normal laboratory conditions were not observed to copulate for a period of 25 days while exposed continuously to temperatures below 59° F. When the temperature was raised above 60°, copulation occurred.

These experiments confirmed field observations, which have indicated that decreasing light intensity inherent in twilight conditions is the normal stimulus for oriental fruit flies to initiate copulation. However, neither continuous light nor continuous darkness inhibited copulatory activity completely. Although both laboratory and field observations have failed to indicate any copulation when the evening temperature is below 59° F., these results suggest that under field conditions the flies may be able to copulate at times when temperatures are favorable in the absence of the normal decreasing light stimulus—for example, in the midafternoon.

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INHIBITION OF THE SUCCINOXIDASE SYSTEM OF SUSCEPTIBLE AND RESISTANT HOUSE FLIES BY DDT AND RELATED COMPOUNDS[1]

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The dehydrochlorination of DDT [2, 2-*bis* (*p*-chlorophenyl)-1, 1, 1-trichloroethane] to DDE [2, 2-*bis* (*p*-chlorophenyl)-1, 1-dichloroethylene] has been shown to occur at a considerably more rapid rate and to a greater extent in resistant than in susceptible strains of house flies (Sternburg *et al.*, 1950a, 1950b; Perry and Hoskins, 1950, 1951a, 1951b; Winteringham *et al.*, 1951). The enzyme system which catalyzes this reaction has been isolated from resistant flies and examined *in vitro* by Sternburg *et al.*, (1953), and the rate of conversion of DDT to DDE *in vivo* has been proposed as a critical yardstick for assessing resistance by Perry *et al.*, (1953). DDT-dehydrochlorinase activity is undoubtedly an important factor in the development of resistance to DDT. However, unchanged DDT in amounts greater than the LD_{50} for susceptible flies can be recovered from living, resistant flies more than 24 hours after topical application of the insecticide. Bradbury *et al.*, (1954) have recently shown that adult flies of a lindane-resistant strain are able to carry over from the larval stage larger amounts of intact lindane (*gamma*-isomer of hexachlorocyclohexane) than are necessary to kill susceptible individuals. The presence of these large amounts of intact insecticides in living, resistant insects indicates that other factors in addition to a detoxication mechanism may be involved in the biochemical mechanisms of resistance. Babers and Pratt (1953) have recently reviewed the evidence in this regard. On the basis of this evidence and their own studies they have concluded that the ability to metabolize DDT is not sufficient explanation for the resistance phenomenon.

In order to ascertain what these other mechanisms may be, as well as to develop information on the possible mode of toxic action of DDT, we have begun an investigation of the effect of DDT and related compounds on important enzyme systems in insects. A study of the effect of DDT and other insecticides on the carbonic anhydrase system of the American cockroach and house fly failed to demonstrate any inhibitory activity of a magnitude that would indicate the involvement of this system in either a resistance or intoxication mechanism (Anderson and March, 1954). On the basis of investigations by Sacktor (1950, 1951) and Johnston (1951) the succinoxidase system of the house fly was selected as promising for further study. Sacktor found that the cytochrome oxidase activity in resistant flies was on the average 50 percent higher than in susceptible flies, while the cyanide-insensitive respiration during the pupal stage was 100 percent higher

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in the resistant strain. Johnston (1951) reported an inhibition of rat-heart succinoxidase and cytochrome oxidase by DDT, DDE, methoxychlor [2, 2-*bis* (*p*-methoxypheny)- 1, 1, 1-trichloroethane], and methoxychlor dehydrochloride [2, 2-*bis* (*p*-methoxyphenyl) 1, 1-dichloroethylene]. Succinic dehydrogenase was not affected. It is the purpose of this paper to present the results of a comparative study of the inhibitory activities of DDT and a number of related compounds, including several which have been shown to be synergistic with DDT against resistant flies although non-toxic alone (March *et al.*, 1952; Speroni, 1952), toward house-fly succinoxidase and its components.

MATERIALS AND METHODS

The highest concentration of succinoxidase in the tissues of the house fly was found in the thorax, presumably in the flight muscles. Enzyme suspensions were prepared by macerating the thoraces of groups of 3- or 4-day-old adult female house flies in distilled water with an all-glass Potter-Elvehjem homogenizer. The crude homogenate was filtered through two layers of gauze or absorbent cotton to remove pieces of cuticle and connective tissue, and the filtrate was diluted to the appropriate concentration with buffer or distilled water. Susceptible (Laboratory strain), DDT-lindane-resistant (Super-Pollard strain), and DDT-synergist-resistant house flies (March *et al.*, 1952) were reared by standard methods in the laboratory, and the adult females which were used for testing were routinely fed on 40 percent sucrose solution.

Succinic dehydrogenase activity was determined colorimetrically with TPTZ (2, 3, 5-triphenyltetrazolium chloride) and neotetrazolium [*p*, *p*^{1}-diphenylenebis-2-(3, 5-diphenyltetrazolium chloride)] according to a procedure modified from Bodine and Fitzgerald (1949) and Glock and Jensen (1953). The components of the reaction mixture in a final volume of 3.0 ml. were: 1.0 ml. 0.1M KH_2PO_4-K_2HPO_4 buffer (pH 7.4), 0.1 ml. 0.02M $AlCl_3$, 0.1 ml. 0.015M $CaCl_2$, 0.3 ml. of 0.01M DDT or other inhibitor in absolute ethanol or acetone, 0.5 ml. fly-thorax homogenate prepared at the rate of eight thoraces per milliliter of distilled water, 0.3 ml. of 0.8M sodium succinate (pH 7.2), and 400 micrograms of neotetrazolium or 800 micrograms of TPTZ. When inhibitors were used, the substrate and indicator were added after 30 minutes preliminary incubation of the other components. For anaerobic experiments, the pyrex ignition tubes in which the homogenates were incubated were placed in a three-liter suction flask which was evacuated to 12–15 cm. of mercury through a stopcock attached to the side arm. All tests were incubated at 35° C. The water-insoluble formazan of the indicator was extracted from the enzyme suspension by shaking the tube contents fifty times with 3.5 ml. of n-butyl alcohol and centrifuging briefly at 946 R.C.F. to separate the two layers. The optical density of the butanol layer was determined spectrophotometrically at 480 (TPTZ) or 520 (neotetrazolium) millimicrons against a solvent blank. The effect of an inhibitor was calculated by comparison with the reduction produced in the absence of inhibitor.

Manometric determinations of cytochrome oxidase, succinoxidase, and succinic dehydrogenase activities were carried out with the Warburg

constant volume respirometer. A strip of filter paper moistened with 0.2 ml. of 2N sodium hydroxide was placed in the center well of each flask. For cytochrome oxidase determinations, each flask contained 1.5 ml. of 0.1M KH_2PO_4–K_2HPO_4 buffer (pH 7.4), 0.3 ml. of 5×10^{-4}M cytochrome c[2], 0.3 ml. 0.2M sodium ascorbate—prepared fresh for each run by neutralizing the appropriate weight of ascorbic acid with 0.2N sodium hydroxide, 0.5 ml. fly-thorax homogenate (0.8 thorax/ml.), 0.1 ml. of absolute ethanol or 0.01M inhibitor in ethanol and distilled water to a volume of 3.0 ml. To measure succinoxidase, the homogenate concentration was increased to 4 thoraces/ml., and the ascorbate was replaced by 0.3 ml. 1M sodium succinate. To determine succinic dehydrogenase, the homogenate concentration was increased to 8 thoraces/ml. and the cytochrome c and ascorbate were

TABLE I

INHIBITION OF SUSCEPTIBLE-STRAIN HOUSE-FLY SUCCINIC DEHYDROGENASE; REDUCTION OF TPTZ BY THORAX HOMOGENATES; 10^{-3}M INHIBITOR

INHIBITOR	% INHIBITION		RELATIVE SYNERGISTIC ACTIVITY*
	AEROBIC	ANAEROBIC	
1. $(p\text{-}ClC_6H_4)_2COHC{\equiv}CH$	88.0	75.7	100
2. $(p\text{-}ClC_6H_4)SO_2NH(p\text{-}ClC_6H_4)$	82.3	68.0	...
3. $(p\text{-}ClC_6H_4)_2COHCH{=}CH_2$	91.4	65.3	40
4. $(p\text{-}ClC_6H_4)_2COHCH_2CH_3$	89.7	64.0	4
5. $(p\text{-}ClC_6H_4)_2CHCl$	68.0	59.2	140
6. $(p\text{-}ClC_6H_4)_2COHCH_3$	36.4	48.3	60
7. $(p\text{-}ClC_6H_4)_2CClCH_3$	36.9	33.8	60
8. $(p\text{-}ClC_6H_4)_2C{=}CCl_2$ (DDE)	62.8	30.7	2
9. $(p\text{-}ClC_6H_4)_2CHCCl_3$ (DDT)	27.7	28.3	1
10. $(p\text{-}ClC_6H_4)_2CHCH_3$	41.2	25.4	100
11. $(p\text{-}ClC_6H_4)_2CH_2$	26.0	19.6	20
12. $(C_6H_5)_2CH_2$	33.9	12.1	...

*March *et al.* (1952).

replaced by 0.3 ml. of 1M sodium succinate, 0.1 ml. of 1×10^{-3}M methylene blue, and 0.3 ml. of 0.01M sodium cyanide. The oxygen uptake of fly-thorax preparations was about one-half that reported for roach muscle by Harvey and Beck (1953), but this may for the most part be due to the presence of non-respiring tissue from cuticle, etc., in the homogenates.

DISCUSSION OF RESULTS

From the results shown in Table I, it is apparent that DDT is not a very effective inhibitor of house-fly succinic dehydrogenase. Since DDT is the only one of the twelve compounds listed in the table that is markedly insecticidal, it seems very unlikely that its inhibitory activity could be involved as a primary factor in the mode of action of DDT. Furthermore, it is evident that there is no positive correlation

[2]Sigma Chemical Co.

between the synergistic activity of the compounds with DDT and their effect upon succinic dehydrogenase. The lower values for inhibition under anaerobic conditions are due to a greater reduction of the indicator anaerobically than aerobically. This increased reduction may be due to decreasing or eliminating competition with naturally occurring hydrogen acceptors.

The results obtained with neotetrazolium, shown in Table II, give somewhat higher inhibitory values under the same conditions than with TPTZ. The relative effectiveness of the inhibitors is in good agreement for both indicators except for DDT, which was considerably more effective when measured with neotetrazolium than with TPTZ. Studies of the responses of the two systems to such inhibitors as cyanide and malonate show that the two tetrazolium salts do not react identically in the system (Anderson, 1954); thus

TABLE II

INHIBITION OF HOUSE-FLY SUCCINIC DEHYDROGENASE; REDUCTION OF NEOTETRAZOLIUM CHLORIDE BY THORAX HOMOGENATES; 10^{-3} M INHIBITOR

INHIBITOR*	% INHIBITION		
	AEROBIC	ANAEROBIC	
	SUSCEPTIBLE	SUSCEPTIBLE	DDT–LINDANE–RESISTANT
No. 1	96.9	....	94.8
No. 2	95.7	94.5	90.6
No. 9. (DDT)	....	93.8	93.5
No. 5	....	92.5	76.1
No. 3	65.7	....	68.3
No. 4	62.4	89.1	87.5
No. 8. (DDE)	40.0	....	37.0
No. 10	15.6	....	0
No. 12	7.4	....	5.3

*See table I.

one might expect such variation. The data demonstrate that there is no real difference in the degree of inhibition of succinic dehydrogenases from susceptible or DDT-resistant strains of house fly by DDT and the more effective inhibitors.

The results from the manometric determinations of succinic dehydrogenase (see table III) are in good agreement with the colorimetric observations, particularly with data obtained with TPTZ since DDT was again less inhibitory than the more active compounds. The results correspond better with the more active compounds (No. 1, 2, 3 and 4 from table I) than with the less inhibitory materials. The inhibition of cytochrome oxidase (see table IV) followed the same pattern as the inhibition of succinic dehydrogenase, but the levels of inhibition were somewhat higher. By this method, as with the colorimetric procedure, no marked differences in sensitivity of homogenates from resistant or susceptible flies were observed. Succinoxidase was more effectively inhibited by all of the compounds examined than either component

of the system alone, as might be expected if the effects are additive. Lindane, like DDT, was not markedly effective toward either component of the system. The most effective inhibitors examined were

TABLE III

INHIBITION OF HOUSE-FLY SUCCINOXIDASE AND SUCCINIC DEHYDROGENASE; OXYGEN UPTAKE BY THORAX HOMOGENATES; 3.3×10^{-4}M INHIBITOR

INHIBITOR*	%INHIBITION				
	SUCCINIC DEHYDROGENASE			SUCCINOXIDASE	
	SUSCEPTIBLE	DDT-LINDANE-RESISTANT	SYNERGIST-RESISTANT	SUSCEPTIBLE	DDT-LINDANE-RESISTANT
G-4	100	....	....	100	.....
No. 2	69.4	61.5	68.1	100	100
No. 1	65.9	65.6	71.6	100	100
No. 3	60.3	57.1	....	.....	.....
No. 4	55.0	....	....	.....	.....
No. 5	.....	59.4	55.2	100	.....
No. 8 (DDE)	18.4	12.4	....	92.8	.....
No. 9 (DDT)	12.2	30.9	38.5	91.4	88.1
No. 10	7.8	....	....	.....	.....
No. 12	8.0	....	....	.....	.....
Lindane	14.5	....	....	.....	.....

*See table I.

TABLE IV

INHIBITION OF HOUSE-FLY CYTOCHROME OXIDASE; OXYGEN UPTAKE BY THORAX HOMOGENATES: 3.3×10^{-4}M INHIBITOR.

INHIBITOR*	% INHIBITION		
	SUSCEPTIBLE	DDT-LINDANE RESISTANT	DDT-SYNERGIST RESISTANT
G-11	99.4	97.8	
G-4	94.5		
No. 2	75.8	78.1	71.4
No. 4	73.2		
No. 1	72.4	75.8	73.8
No. 3	72.1	....	65.7
No. 8 (DDE)	71.4	....	54.4
No. 7	69.6		
No. 9 (DDT)	67.9	68.5	57.2
No. 10	59.4	....	57.2
No. 12	58.2		
No. 5	39.0	....	65.8
Lindane	33.0	9.5	15.8

*See table I.

the bisphenolic compounds 2, 2′-methylenebis-(3, 4, 6-trichlorophenol) (G-11) and 2, 2′-methylenebis-(4-chlorophenol) (G-4). These were found by Gould *et al.* (1953) to be good inhibitors of glucose, lactic

and succinic dehydrogenases and cytochrome oxidase of the bacteria *Bacillus subtilis* and *Escherichia coli*. The data in tables III and IV support the conclusions drawn from the data in table I, that there is no correlation between inhibition, synergistic activity, and *in vivo* toxicity.

Slater (1949) defined the characteristics of non-specific inhibitors which act physically by absorption on the enzyme particles or by dispersing or aggregating the reactants. One of the criteria for this type of inhibition of succinoxidase was that the system be inhibited completely by concentrations of the inhibitor which would partially inactivate cytochrome oxidase and only slightly inhibit succinic dehydrogenase. An inspection of the tables shows that these conditions are obtained for the less effective inhibitors such as DDT and DDE.

Johnston (1951) reported that DDT and DDE inhibited rat-heart succinoxidase and cytochrome oxidase but not succinic dehydrogenase. The data presented here show moderate inhibition of cytochrome oxidase by DDT and DDE and slight inhibition of succinic dehydrogenase, but nearly complete inactivation of succinoxidase. This more effective inactivation of succinoxidase may indicate that the effect of DDT and DDE is largely physical according to Slater's definition.

Abood and Gerard (1953) reported the inhibition of rat-heart cytochrome oxidase and *Salmonella aertrycke* succinoxidase, which is lacking in cytochrome c and cytochrome oxidase, by compounds having a diphenyl nucleus. The monophenyl and tri-phenyl compounds examined were without effect. They concluded that chemical combination of the diphenyl compounds with enzymes is unlikely, and that solution of the inhibitors in the mitochondrial phospholipids might be responsible for the inactivation. The structural similarities of the compounds studied here might lead one to expect that they would have relatively similar physical effects; however, it is difficult to correlate on this basis the wide range of inhibitory activity which they produced. The greater activities of several of the more inhibitory compounds suggests that their mode of action may be due to a stereochemical affinity for certain enzymes or enzyme complexes, which depends for its effect upon the nature of both the aromatic and aliphatic substituents.

DDT and the other compounds investigated here resemble the bisphenolic inhibitors examined by Gould *et al.*, (1953) in structure, activity, lack of specificity and absence of positive correlation between *in vitro* inhibition and *in vivo* toxicity. This may indicate that a similar mode of action is responsible for the inhibitory properties of both types. Gould and coworkers found that cultures of *E. coli* flourished on media containing several times the concentration of G-11 necessary to inhibit the growth of *B. subtilis* cultures completely although for both species several dehydrogenases and cytochrome oxidase were inhibited *in vitro* to the same extent. G-4 and G-11 were the most effective inhibitors of those examined for house-fly succinoxidase and its components; however, they were not toxic to susceptible and resistant flies *in vivo* and did not act as synergists with DDT against

resistant flies. They were found to inhibit house-fly acetylcholinesterase 90 percent at 1×10^{-3}M.

The relative ineffectiveness of DDT as an inhibitor, its similarity in activity to non-toxic DDE, and the lack of correlation between *in vitro* inhibition and *in vivo* toxicity for the most effective inhibitors indicate that the inhibitory activity reported here is not a factor of primary importance in the mode of action of DDT and the DDT-synergists or in the mechanism of DDT-resistance in the house-fly.

SUMMARY AND CONCLUSIONS

1. The effect of suspensions of DDT, DDE, and related compounds on the *in vitro* activity of the succinoxidase system and its components in house flies was determined colorimetrically and manometrically. These compounds were found to inhibit cytochrome oxidase and succinoxidase, and to a lesser degree dehydrogenase.

2. No pronounced differences in sensitivity to DDT and related compounds were observed between enzyme preparations from resistant and susceptible strains of flies.

3. The inhibition of succinoxidase and its components by DDT is not a primary factor in the mode of action of DDT and the DDT-synergists or in the mechanism of DDT-resistance in the house-fly.

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CATALOGUE ILLUSTRÉ DES LUCANIDES DU GLOBE, by R. DIDIER ET E. SÉGUY. 223 pp., 136 figs., with atlas of 112 plates (903) figs.). Paul Lechevalier, Paris, 1952–1953. Price, 8000 francs.

ATLAS DES COLÉOPTÈRES LUCANIDES DU GLOBE, GENRE LUCANUS, by ROBERT DIDIER. 20 plates (185 figs). Paul Lechevalier, Paris, 1949.

The Lucanidae are among the largest and most attractive of the Coleoptera. 356 species and 65 varieties in 45 genera were listed for the world by Gemminger and von Harold in 1868. Forty-two years later (1910) van Roon cited 750 species and 33 varieties in 77 genera; and now, after another forty-three years, the present *Catalogue* records about 1100 species and 150 varieties in 106 genera with excellent figures of about 230 species and varieties. If the 19 species and varieties of the 1949 *Atlas*, which are not duplicated in the 1952–53 publication, are included, this number is raised to about 250. Perhaps the Lucanidae are a conspicuous enough group so that the knowledge of the world fauna is beginning to attain some degree of completion.

The catalogue portion of this book (pp. 69–223) lists the genera in systematic order and the species (including fossil species) under each genus alphabetically. Basic references, references supplemental to the 1910 van Roon catalogue, and geographical distribution are given for each species and variety. Pages 23 to 68 contain taxonomic notes and descriptions of new species, and pages 7 to 22 are introductory. The students of these beetles are confronted by an extraordinary sexual dimorphism and a great variation in the body size and structure of the mandibles in the male. Leuthner's (1885) nomenclature (*forma priodonta, f. amphiodonta, f. mesodonta, f. telodonta*) and an additional *forma anisodonta* are employed in describing this variation in the mandibles and in many instances both sexes and series of variously developed males of the same species are figured.

The *Catalogue Illustré* constitutes Parts XXVII and XXVIII of Lechevalier's *Encyclopédie Entomologique*, which has included over the past twenty years a most notable series of coleopterological books. It and they are a continuing indication of the ascendancy of the French in the present day study of beetles.

—MELVILLE H. HATCH

THE EFFECT OF TEMPERATURE AND FOOD ON THE DEVELOPMENT OF THE VARIEGATED CUTWORM PERIDROMA MARGARITOSA HAW. (ORDER LEPIDOPTERA, FAMILY NOCTUIDAE)[1, 2]

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One of the commonest and most widely distributed cutworms is the variegated cutworm which has been reported as attacking a wide variety of plants throughout the world. Ordinarily it is a minor pest, but occasionally it occurs in enormous numbers and destroys every plant in its path. There are numerous records of crops attacked and damage done by the variegated cutworm. Aside from field observations, however, little has been written on its biology. This paper is concerned with certain reactions of the larva and pupa to temperature and food.

The rearing stock of cutworms was started in August 1948 from a small group of mature larvae obtained in the field, and was maintained with only one revitalization throughout the course of the experiments, a period of almost three years. Larvae were reared in an insectary and other rooms and in cabinets where the temperature and humidity were constant. They were kept individually in half-pint cottage cheese cartons each covered with half of a Petri dish. Plant material used as food was kept fresh by wrapping the base of each sprig in a moist wad of absorbent cotton and forcing it into the mouth of a water-filled vial or flask. The vial or flask was then laid on its side, and as long as there was any water in it, the cotton plug was kept moist and the sprig was provided with moisture. This method was illustrated by Peterson (1947, Pl. 19—2). No attempt was made to segregate the moths emerging from the several experiments. All were used to maintain breeding stock and were placed in wide-mouth gallon jars covered with paper towelling for the purpose of mating and oviposition. Eggs, which were laid in masses of up to several hundred on the towelling, were collected periodically and sterilized externally by soaking for two hours in 10 percent formaldehyde. In each experiment larvae from a single egg mass were used.

As is noted later in this paper, the developing larvae of the variegated cutworm are very sensitive to variations in the freshness and

[1]According to a private communication from J. G. Franclemont the scientific name of this insect should be *Peridroma saucia* Hbn. since it was Hubner, who first described it some time between 1803 and 1808.

[2]The author wishes to acknowledge the generous assistance of Professors A. E. Michelbacher, E. O. Essig, and W. W. Middelkauff of the Department of Entomology and of Professor E. A. Steinhaus, Department of Biological Control, University of California, in the preparation of this material.

succulence of their food. For this reason, an effort was made to conduct any given series of experiments within as brief a period as possible to minimize the effects of this variable. For example, the feeding experiments summarized in Tables III and IV were conducted in

TABLE I

NUMBER OF FIRST INSTAR LARVAE OF *Peridroma margaritosa* HAW. SUCCESSFULLY PUPATING AND REACHING ADULT WHEN REARED AT VARIOUS CONSTANT TEMPERATURES ON FRESH MATURE ALFALFA

TEMPERATURE IN DEGREES CENTIGRADE	1ST INSTAR LARVAE	PUPAE	ADULTS
15	55	15	12
20	63	24	22
25	50	29	23
29	57	25	25
32	52	21	6

TABLE II

EFFECTS OF LOW TEMPERATURES ON THE DEVELOPMENT OF EGGS AND LARVAE OF *Peridroma margaritosa* HAW.*

EARLY INCUBATION PERIOD		LATE INCUBATION PERIOD		LARVAL PERIOD	
Temperature in Degrees C	Length of Early Incubation	Temperature in Degrees C	Hatching of Eggs	Temperature in Degrees C	Development of Larvae
0	over 1 week	0	Failed	..	....
0	over 1 week	25	Failed	..	..
0	less than 1 week	25	Hatched	25	Developed
2.5	over 3 weeks	25	Failed	..	..
2.5	less than 3 weeks	25	Hatched	25	Developed
5.0	over 3 weeks	25	Failed	..	..
5.0	less than 3 weeks	25	Hatched	25	Developed
*8.0	over 1 week	8	Hatched	8	Died in 2–3 weeks
8.0	12–20 days	25	Hatched	25	Developed
15.0	over 1 week	15	Hatched	15	Developed
25.0	3–4 days	25	Hatched	5	Died in 2 weeks

*50 to 100 eggs were used in each test.

the summer of 1950 during a period of less than 3 months. Larvae in the temperature experiments summarized in Tables I and Figures 1, 2, and 3 were hatched from a single egg mass in the spring of 1951.

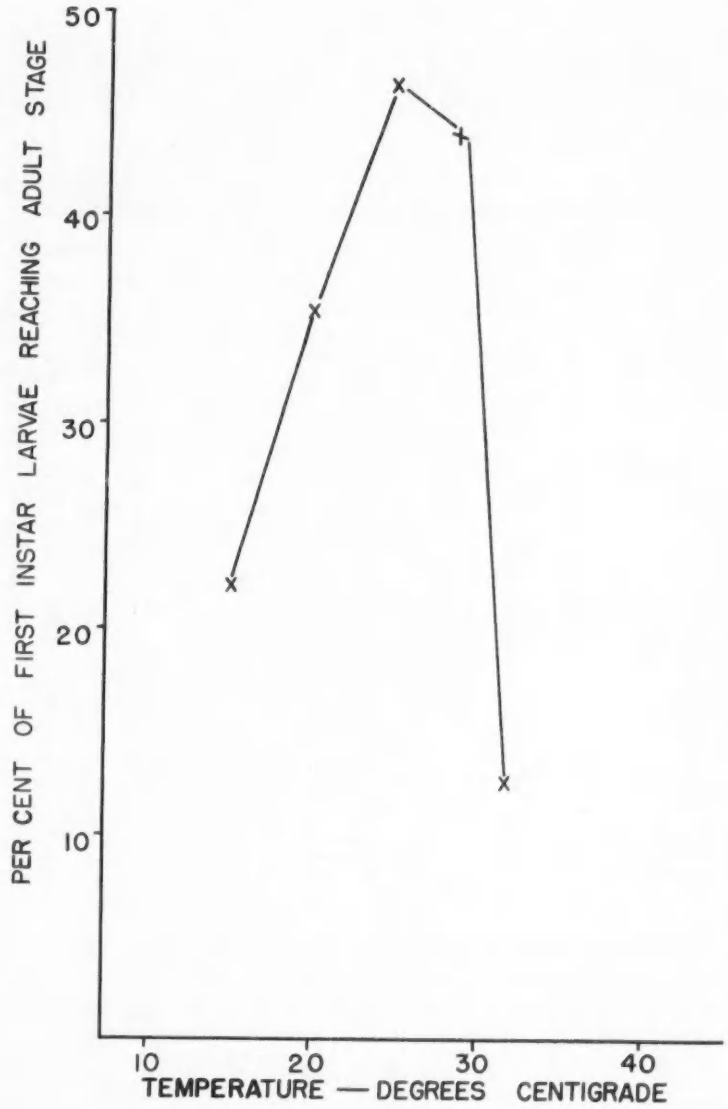

FIG. 1.—Percent of first instar larvae of *Peridroma margaritosa* Haw. reaching adult stage when reared at various constant temperatures on fresh mature alfalfa. Each temperature group contained at least 50 larvae.

An illustration of the importance of brevity in experiments may be noted in Figure 3. The period required for development of larvae at 15° C was so great that alfalfa available for the experiment increased in succulence. This resulted in a 15–20 mg. increase in average pupal weight over that which might have been expected on the basis of other experiments.

TABLE III

RELATIVE ATTRACTIVENESS OF VARIOUS HOSTS TO NEWLY HATCHED LARVAE OF *Peridroma margaritosa* HAW.

HOST	ATTRACTED TO HOST		HOST	ATTRACTED TO HOST	
	Number	Percent		Number	Percent
Alfalfa	1868	84	Alfalfa	416	74
Tobacco	200	9	Bean leaf	146	26
Grape leaf	147	7			
Alfalfa	583	61	Alfalfa	495	80
Spinach	333	35	Banana squash leaf	80	13
Red beet leaf	35	4	Kale	49	7
Alfalfa	575	66	Alfalfa	317	62
Grass	229	27	Lettuce	194	38
Mustard	60	7			
Alfalfa	189	52	Alfalfa	197	65
Turnip leaf	122	34	Tomato leaf	89	29
Carrot leaf	48	14	Strawberry leaf	17	6
Alfalfa	829	79	Bean leaf	809	54
Grass	159	15	Citron leaf	445	30
Corn leaf	54	6	Kale	249	16
Spinach	885	72	Turnip leaf	640	59
String bean	211	17	Carrot leaf	394	36
Corn leaf	135	11	Red beet leaf	58	5
Banana squash leaf	806	64	Lettuce	646	52
Tomato leaf	293	23	Cabbage	327	26
Strawberry leaf	170	13	Celery	281	22

EFFECT OF TEMPERATURE ON LARVAL AND PUPAL DEVELOPMENT

A series of experiments was conducted in which the cutworm was reared at various constant temperatures using fresh succulent alfalfa as food. Although several hundred larvae were reared at temperatures below 20° C. and one group at a temperature ranging between 10 and 15° C. diapause was never encountered. The results of temperature experiments are shown in Table I, and Figures 1, 2, and 3. In these experiments it was noted that larvae in certain groups had a seventh instar. With these larvae, the sixth instar tended to be shorter and the size of individuals smaller than when the larvae had only six instars. It is seen from Figure 2 that the period necessary for

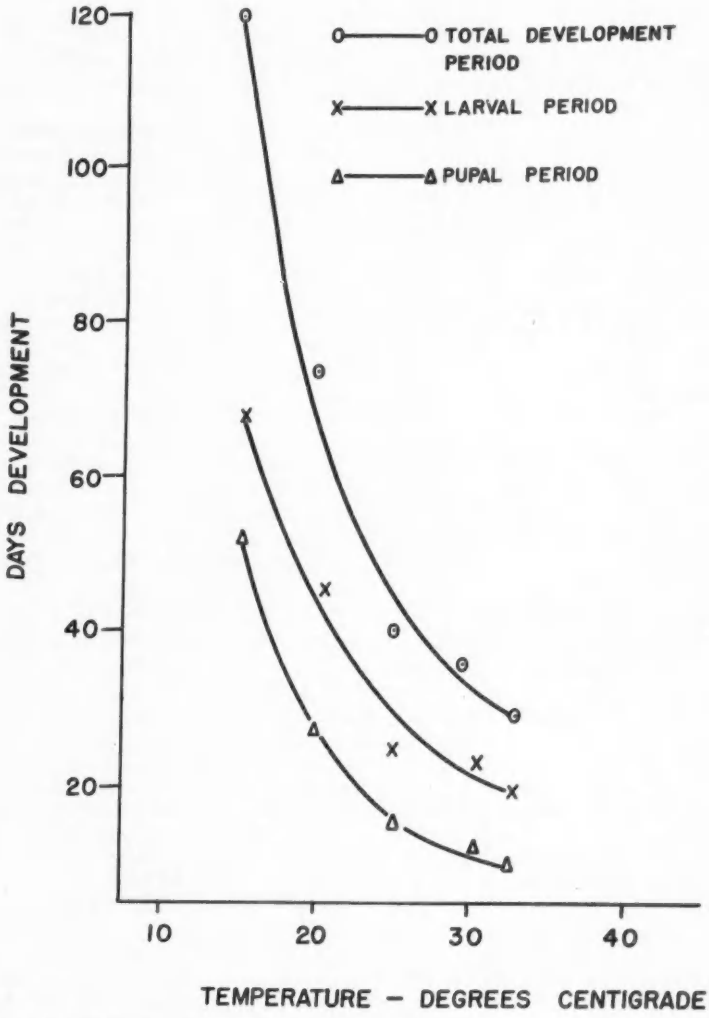

FIG. 2.—Effect of various constant temperatures on the average duration of the larval and pupal periods of *Peridroma margaritosa* Haw. when reared on fresh mature alfalfa.

development decreased from 15 to 32° C. However, it should be noted (Figure 3) that the lightest pupae were obtained at 32° C. Also at the highest temperature the mortality rate for pupae was very high, only 12 percent of the individuals surviving to adult. This indicated that this temperature was beyond the optimum despite the fact that the larvae were the largest and larval development was the most rapid.

At 0° C. eggs slowly turned pink if fertile, such a process requiring several weeks. However, they failed to develop beyond this stage unless removed to a warmer environment within a week after being placed at 0° C. At 2.5° C. and 5° C. fertile eggs turned pink in two or three days but did not develop beyond this stage unless removed to a warmer environment within two or three weeks. Larvae from eggs incubated at 25° C. and then placed at 5° C. died in two weeks without apparent feeding. At 8° C. eggs hatched in approximately twenty-five days, but the larvae died in two or three weeks unless removed to a higher temperature. The lower threshold of development occurred between 8 and 15° C. Individuals reared from eggs incubated for 12 and 20 days at 8° C. and then held at 25° C. developed in a normal manner. The above results are summarized in Table II. There was no significant difference either in the length of developmental period or in the pupal weight between these larvae and those from eggs which were incubated at room temperature. Cutworms reared at constant temperatures from 15 to 32° C. completed development, and the optimum temperature for development based upon general activity, low mortality, weight, and vigor lay close to 25° C.

EFFECT OF FOOD ON LARVAL AND PUPAL DEVELOPMENT

Dethier (1941) stated that certain chemical constituents in plants, probably essential oils, make them more or less attractive to insects, and when given a choice, an insect will select certain species or groups of plants for feeding. The variegated cutworm is no exception to this rule. Although it is not as exacting as many insects and during a severe outbreak will consume almost any type of plant material[3] it appears to prefer alfalfa or related legumes to most other plants.

During the present research, a series of experiments was conducted to determine the relative attractiveness of various plants to newly emerged variegated cutworms. In most tests, materials were used immediately after harvesting, and care was taken to use only vigorously growing, unsprayed leaves. Hatching egg masses were placed in the center of a 2′ x 2′ shallow wooden tray and surrounded by a circle of several dozen plant sprigs prepared in the manner describeed earlier in this paper. Two or three kinds of host material were used in each test, and the sprigs were alternated to compensate for any phototropism or other reaction which would tend to draw the larvae toward any particular part of the tray. After twelve hours when larvae had crawled to the sprigs, and before the sprigs had dried, they were removed and the larvae counted on each plant type. An

[3]Ninety-nine species of plants, representing a wide range of botanical groups, have been reported as attacked by the variegated cutworm.

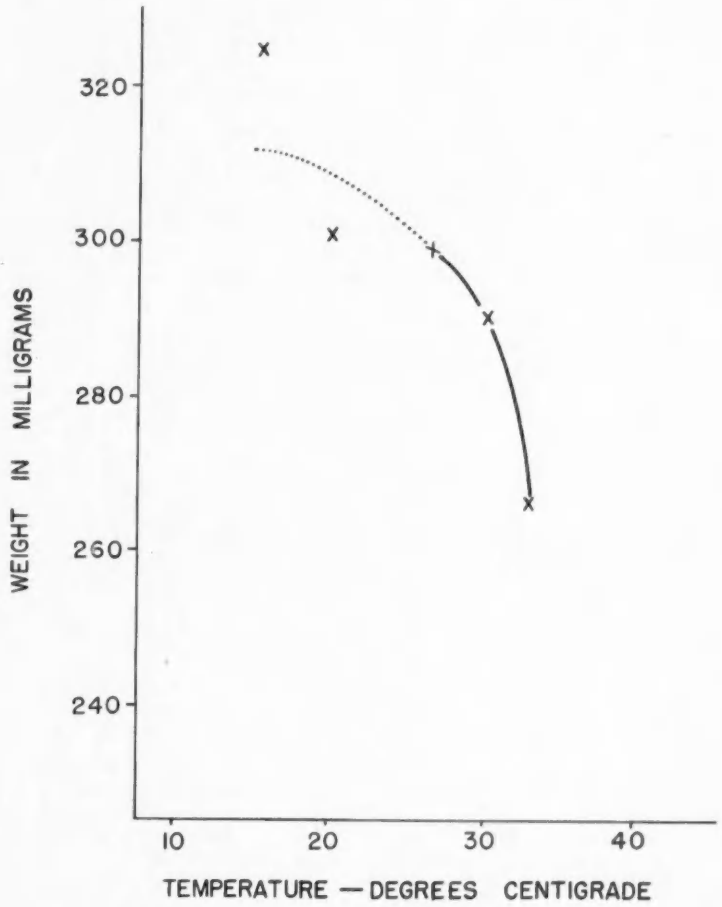

FIG. 3.—Effect of various constant temperatures on the average pupal weight the day after pupation of *Peridroma margaritosa* Haw. when reared on fresh mature alfalfa. The discrepancy at 15 C. was caused by the unavoidable use of more succulent alfalfa at the end of the extremely long rearing period.

effort was made to include alfalfa in as many tests as possible, so that there would be a standard for comparison of results. The information obtained is summarized in Table III. It should be noted that larvae showed an outstanding preference for alfalfa. The larvae were little attracted to kale, or to string bean although, as will be seen later, they developed very well on both these hosts. The results obtained were in general agreement with other information noted in the present research, and with field observations reported by other authors.

TABLE IV

NUMBER OF INDIVIDUALS OF *Peridroma margaritosa* HAW. SURVIVING DURING THE SEVERAL INSTARS WHEN REARED ON VARIOUS HOSTS AT 25° C

HOST	INDIVIDUALS SURVIVING AT BEGINNING OF EACH INSTAR									PERCENT REACHING ADULT
	LARVA						PRE-PUPA	PUPA	ADULT	
	1st	2nd	3rd	4th	5th	6th				
Celery	47	39	32	28	26	25	19	18	17	36
Alfalfa	427	291	229	206	198	192	176	155	147	34
Kale	129	62	55	52	51	50	50	44	43	33
Cabbage	108	70	64	64	63	50	46	42	32	30
Lettuce*	...	48	41	41	41	38	24	23	14	29
String-bean	166	115	88	85	84	77	64	50	45	27
Tomato leaf	77	37	28	26	26	24	22	21	21	27
Bean leaf	186	127	104	101	97	85	48	43	40	21
Citron leaf	67	36	27	24	21	19	16	13	12	17
Corn leaf	67	36	23	22	19	14	10	9	9	16
Tobacco	64	34	30	26	26	26	26	9	9	14
Spinach	126	67	40	29	19	17	17	13	12	9

*First instar not observed.

In the present studies, it was noted that both the type and the quality of food affected the development of larvae. Although many investigators have reported this species as attacking a wide range of hosts it is probable that normal development is completed on relatively few species of plants, and that others are consumed only when the primary hosts are not available. Waters (1937) believed that plants of the Beet Family (Chenopodiaceae) were the most suitable for insectary rearing, while Wymore[4] believed that the cutworms preferred crucifers such as collards. The present author was unable to discover any particular botanical group on which the variegated cutworm showed superior development, although alfalfa, string bean, and kale were consistently favorable hosts. In the present experiments alfalfa was the principle food used mainly because the insect thrived on it and it was the most available plant throughout the year. How-

[4]Private communication to Dr. E. A. Steinhaus.

ever, as will be seen in the following discussion, alfalfa probably was not as suitable for larval development as certain other hosts.

The suitability of the various hosts for rearing the cutworm was judged by the following three criteria: (1) relative mortality of larvae and pupae (Table IV); (2) relative duration of the larval and pupal periods (Table V); and (3) relative weight of the pupae the day after pupation[5] (Table VI).

Records were kept of the percentage of variegated cutworms reaching the adult stage on several hosts. The information obtained is given in Table IV. It should be noted that the percentage survival on the first seven hosts in the table was somewhat similar, indicating that as far as survival was concerned these hosts were about equal.

TABLE V

DURATION OF THE LARVAL AND PUPAL PERIODS OF *Peridroma margaritosa* HAW. WHEN REARED ON VARIOUS HOSTS AT 25° C

HOST	NUMBER OF INDIVIDUALS SURVIVING TO ADULT	DAYS IN EACH STAGE						STANDARD ERROR OF THE MEAN
		LARVA		PUPA		TOTAL LARVA & PUPA		
		Range	Average	Range	Average	Range	Average	
Kale	31	21–26	22.2	14–17	15.1	35–43	37.3	±0.3
Bean leaf	17	22–24	23.0	13–18	15.3	37–40	38.3	±0.3
String bean	17	23–25	24.3	13–19	15.1	36–44	39.4	±0.7
Alfalfa	18	24–30	26.9	13–16	14.8	37–46	41.7	±0.8
Carrot leaf*	20	.....	....	.....	....	39–46	42.0	±0.5
Spinach	12	25–31	27.8	14–16	14.8	39–47	42.6	±0.6
Tobacco	9	26–30	28.5	14–16	15.2	40–46	43.7	±0.7
Turnip leaf*	13	.....	....	.....	....	42–48	45.2	±1.0
Cabbage	32	29–40	31.5	13–19	15.7	42–59	47.2	±0.8
Citron leaf	11	26–37	31.9	14–20	16.7	40–57	48.6	±1.4
Tomato leaf	21	34–40	34.6	14–20	16.2	46–60	50.8	±1.0
Celery	17	33–40	35.4	14–18	15.8	47–58	51.2	±0.8
Lettuce	14	38–47	40.2	13–16	15.0	51–63	55.2	±0.8
Corn leaf	9	41–51	44.0	15–16	15.8	56–67	59.8	±1.0

*Detailed observations not made in these experiments.

The highest mortality occurred among larvae of the first two instars where approximately half of them died. During the third to fifth instars few deaths occurred. However, there was an increase in mortality during the final larval and prepupal stages. Most individuals that successfully pupated emerged.

A series of experiments was conducted in which the length of development of larval and pupal periods on several hosts was compared. The information obtained is summarized in Table V. The time required for completion of the larval and pupal stages varied from 37

[5]Pupae of the variegated cutworm average about a 12 percent loss of weight during the pupal period, and therefore they were weighed the day after pupation to obtain standard results.

days on kale to 60 days on corn leaves. Information in Table V indicates that while food exerted a definite influence on the length of larval development, it had little effect on the duration of the pupal period.

The information on pupal weights is summarized in Table VI. With the exception of kale, string bean, tobacco and corn leaf, there was little correlation between the size of individuals and the duration of the larval and pupal periods. It is interesting to note that while individuals reared on string bean were the largest, those reared on the leaves of the same species of plant were among the smallest. In certain experiments, detailed records were kept of the length of larvae during the several instars. These records indicate that the effect of food on the size of individuals was most pronounced during the final

TABLE VI

PUPAL WEIGHT THE DAY AFTER PUPATION OF *Peridroma margaritosa* HAW. WHEN REARED ON VARIOUS HOSTS AT 25° C

HOST	NUMBER OF INDIVIDUALS SURVIVING TO ADULT	WEIGHT IN MG		
		RANGE	Average	Standard error of the mean
String bean	17	357–737	480.0	± 8.9
Kale	31	399–560	479.0	± 6.9
Celery	17	331–512	447.2	±13.7
Cabbage	32	375–492	418.7	± 6.1
Tobacco	9	332–454	390.0	±14.0
Tomato leaf	21	300–412	362.0	±24.5
Alfalfa	18	259–446	356.5	±11.0
Lettuce	14	223–479	355.3	±19.0
Carrot leaf	20	264–431	338.8	±11.0
Turnip leaf	13	209–416	327.3	±16.0
Citron leaf	11	219–443	310.4	±22.0
Bean leaf	17	241–421	309.2	±12.5
Spinach	12	247–366	304.1	±11.3
Corn leaf	9	148–296	231.7	±17.0

instar, the differential effect being insignificant during the earlier instars. This is in contrast to the variation in number of days in each larval period which was equally manifest during the entire larval development.

Not only the kind of food but also its condition is important in influencing larval development. After a plant has been picked it undergoes a number of chemical changes, and these apparently are reflected in the development of the insect feeding on the plant. It was noted that on several occasions when food that was not fresh was fed to larvae, smaller individuals resulted. Furthermore, even though fresh plant material was used in feeding larvae, it still varied in quality. An example of this was the alfalfa which was used on many occasions during the present series of experiments. During the fall and winter when growth was slow, the leaves tended to be small, dry, and hairy. With the advent of warmer weather the leaves,

in contrast, were large, succulent, and with few hairs. As these plants matured during the summer, the leaves were somewhat less succulent than the spring growth, but were more succulent than the dry winter growth. The variation in succulence of the alfalfa was reflected in the pupal weight, and the more succulent leaves produced the heavier pupae.

SUMMARY

In a series of controlled experiments it was demonstrated that the variegated cutworm *Peridroma margaritosa* Haw. was influenced in its development by both temperature and the quality of food.

No diapause was encountered during experiments in which larvae were reared at temperatures ranging between 10 and 15° C. Development of the cutworm occurred between constant temperatures ranging from 15 to 32° C. Between these temperatures the developmental period decreased with an increase in temperature, although above 25° C. the decrease was not pronounced. At temperatures below the optimum (20 to 25° C.), pupal weights were approximately the same as at the optimum, but above the optimum, pupal weights decreased rapidly, and adults often failed to emerge.

Where given a chance to select a host, newly hatched larvae most frequently went to alfalfa. Hosts were compared at a constant temperature of 25° C. Their suitability was judged by: (1) relative mortality of larvae and pupae; (2) relative duration of development; and (3) relative weight of pupae. Hosts such as string bean, kale, and alfalfa were found to be the most favorable. Among the poorer hosts were lettuce, tomato leaf, and corn leaf. However, the condition of the host exerted a marked influence and resulted in wide variations in the rate of development. With any given host, development was best where the food was fresh and succulent.

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INSECTOS DO BRASIL. 8.º TOMO. COLEOPTEROS, 2.ª PARTE, by by A. DA COSTA LIMA. Pp. 323, 259 figs. Escola Nacional de Agronomia, Série Didàtica No. 10. 1953.

This is a continuation of Dr. Costa Lima's great work on the insects of Brazil. This is a continuation of Dr. Costa Lima's great work on the insects of Brazil. The present volume, the second on the Coleoptera, takes up the superfamilies Scarabaeoidea, Dascilloidea, Byrrhoidea, Dryopoidea, Elateroidea, Cantharoidea, Lymexyloidea, Cleroidea, Bostrichoidea, Nitiduloidea, Cucujoidea, Derodontoidea, Colydioidea, and Coccinelloidea. The treatment is similar to that of preceding volumes of the series.—M.T.J.

A REVIEW OF THE GENUS HETEROTHRIPS HOOD (THYSANOPTERA: HETEROTHRIPIDAE) IN NORTH AMERICA, WITH DESCRIPTIONS OF TWO NEW SPECIES

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Since the genus *Heterothrips* was erected by Hood in 1908 over forty species have been described. Of the presently recorded species about half are North American. In this review we are concerned only with those forms in North America including Mexico.

As is often the case in such work, it has not been possible to examine all of the types. We have not seen specimens of Hood's species *lasquerellae* or *vernus*. Therefore, the exact status of all of the species could not be determined as accurately as hoped. We wish to thank R. S. Bigelow, Ottawa, Canada; George Mack, Director, Queensland Museum; Kellie O'Neill, E. S. Ross, A. F. Shull and the Michigan Museum, L. J. Stannard, and A. N. Tissot for the loans and gifts of specimens and their cooperation in making this review as nearly complete as possible.

In studying the collection data on specimens of this genus one immediately notes that practically all specimens are taken in the spring of the year. We (Bailey, 1940) reared one species (*vitifloridus*, n. sp.) from wild grape flowers and determined that it formed a cocoon in the topsoil and plant debris beneath the host. The presence of the tarsal claw in all species examined, together with the spring and early summer appearance of the active forms, indicate only one generation a year. *Heterothrips* is similar to the aeolothripoids in this one respect. But, to our knowledge, they are plant feeders only whereas the aeolothripoids are preferentially predaceous in habit.

It was thought at one time that the species of this group were narrowly restricted in their hosts, even specific. An examination of the host records for *aesculi*, *auranticornis* and *pectinifer*, for example, indicate that the adults at least feed on many plants. Some species on the other hand, such as *arisaemae* and *salicis*, appear to be quite restricted. At present, the genus is found only in the Western Hemisphere. It is interesting to note that, with the exception of *watsoni*, n. sp., none of the North American species are found in South America and vice versa.

The synonymy of the genus was given by Bagnall (1911) and Hood (1913). Since that time many new species and the subgenus *Protemnothrips* Hood, 1937, have been described.

Heterothrips Hood

Heterothrips Hood, 1908 (*nec Heterothrips* Buffa, 1908), Bull. Ill. State Lab. Nat. Hist. 8:361.(Aug. 22).
Phyllothrips Buffa, 1908, (*nec Phyllothrips* Hood, 1908), Redia 5:123. (Dec. 5).
Phyllothripsiolus Strand, 1912, Int. Ent. Zeitschr. 5 (40): 287.
Heterothrips Bailey, 1949, Fla. Ent. 32(1):22.

Antennae nine-segmented; segments three and four barrel-shaped or vasiform with a circumpolar band of sensoria arranged in single or multiple rows, basal portion of third with constrictions. All segments free except in subgenus *Protemnothrips* Hd., in which the three terminal segments are fused. Head usually wider than long. Ocelli present. Maxillary palpi three-segmented. Labial palpi two-segmented. Legs slender, fore femora somewhat enlarged in both sexes, second segment of fore tarsi in both sexes with a finger-like hook at base. Forewings slender and pointed, the two longitudinal veins set with short, stout bristles. Posterior margin of abdominal segments fringed with small setae variously arranged, the bases of some groups fused into plates. The sides of the abdominal segments are reticulated along which markings are usually arranged rows of microtrichia as in *Sericothrips* Hal. Ovipositor curved downwards.

Male smaller than female with abdominal fringe of setae weaker. Sternites of abdominal segments II, III, or IV to VIII usually with a clear oval area on each, centrally and anteriorly located. Some species with a pair of heavily chitinized finger-like projections on dorsum of segments IX; others with neither projections or oval areas.

Type of the genus: *Heterothrips arisaemae* Hood, 1908. By monotypy.

The affinities of this genus are with the aeolothripoids in respect to the number of antennal segments, the sensoria, the claw on the fore tarsi and in their seasonal life history. However, the down-curved ovipositor, wings and palpi indicate a close relationship with the thripoids. In 1912 Bagnall established the family Heterothripidae for this genus, "on account of the structure and segmentation of the antennae, the character of the sensoria, and the tarsal appendages." Karny (1923), Watson (1924), Moulton (1932), and Priesner (1949) have retained it as a useful grouping which today also includes the non-fossil genera *Adiheterothrips* Ram., *Fauriella* Hd., *Holarthrothrips* Bagn., *Oligothrips* Moult., and *Opisthothrips* Hd.

Two species, other than those presently included, have been described in the genus *Heterothrips* Hood. *H. dubius* and *keatsii* of Girault, 1926, were referred to *Anaphothrips* by their author in a subsequent paper (1928). Girault's types, deposited in the Queensland Museum, Brisbane, Australia, were examined by us, and we find they are not *Heterothrips*. Because of their poor condition we are unable to place them more accurately.

KEY TO *HETEROTHRIPS* SPECIES OF NORTH AMERICA, INCLUDING MEXICO

1. Comb fringe of setae on posterior lateral margins of abdominal terga simple and not fused into plates at base....(Group I)..... 2
   Comb or fringe of setae on posterior lateral margins of abdominal terga fused into plates at base.....(Group II) 7
2 (1). Pronotum finely cross-striate (Plate II, fig. 4)..... 3
   Pronotum reticulate, often faintly so, lines tending to form polygons (Plate II, fig. 12)..... 4
3 (2). Middle and hind tibiae pale yellow at both ends. Antennal segment III 0.072 mm. long; V grayish yellow.....**analis**
   Middle and hind tibiae yellowish brown at distal ends only. Antennal segment III 0.054–0.061 mm long; V brown.....**vitis**

4 (2). Sensoria on tip of antennal segment III (and sometimes IV) reduced to a narrow single row on dorsum (Plate I, fig. 3) ..... **cuernavacae**
Sensoria on antennal segments III and IV arranged in two or more rows 5
5 (4). Sensoria arranged in more than two rows (Plate I, fig. 17) ..... **watsoni**
Sensoria arranged in two rows (Plate I, fig. 1) 6
6 (5). Antennal segments III and IV yellow usually with orange pigment in distal portion. Middle and hind tibiae yellow at both ends. **auranticornis**
Antennal segments III and IV light brown, III yellow in basal two-thirds. Legs brown ..... **gillettei**
7 (1). Pronotum cross-striate with lines not closely parallel (Plate II, fig. 3) ... 8
Pronotum smooth, or reticulated to varying degrees, frequently forming polygons (Plate II, figs. 11, 15) 10
8 (7). Terminal comb setae on abdominal plates two or three times longer than base. One row or circle of sensory areas at tip of antennial segments III and IV ..... **vernus**
Terminal comb setae on abdominal plates shorter than base. Two irregular rows or circles of sensory areas, at least ventrally, at tip of antennal segments III and IV 9
9 (8). Antennal segment III white to yellow; 0.076–0.087 mm. in length. Sides of comb plates overlapping and appearing to be fused (Plate V, fig. 7). Male glandular areas small (0.025–0.028 mm. wide on segment VIII) (Plate IV, fig. 6) ..... **prosopidis**
Antennal segment III with basal two-thirds yellow, remainder dark gray-brown; 0.071 mm in length. Male glandular areas large (0.060 mm. wide on segment VIII) ..... **lasquerellae**
10 (7). Comb plates extending across entire posterior margins of terga of abdominal segments II–V (Plate V, fig. 8) ..... **limbatus**
Comb plates only on posterior lateral margins of abdominal segments I-VI (Plate III, fig. 3; Plate V, fig. 5) 11
11 (10). Base of comb plates completely fused (Plate III, fig. 3). Antennal segment III 0.090 mm. long. Glandular area on sternite of abdominal segment VIII of male wide, 0.065 mm. in width (Plate IV, fig. 4) ..... **decacornis**
Base of comb plates partially fused, overlapping, or distinct. Antennal segment III shorter than 0.090 mm. Glandular areas on sternite VIII of male absent or smaller 12
12 (11). Terminal abdominal segment (X) long and pointed (Plate V, fig. 4), 0.1–0.11 mm. in length 13
Terminal abdominal segment not long and pointed (Plate V, fig. 2) 14
13 (12). Pronotum (Plate II, fig. 10) and abdominal sternites heavily setose. Abdominal sternites with posterior fringe of setae at lateral margins only (Plate IV, fig. 16) ..... **mexicanus**
Pronotum and abdominal sternites not heavily setose. Abdominal sternites II–VI with complete fringe of setae on posterior margins (Plate IV, fig. 2) ..... **pectinifer**
14 (12). Antennal segments III and IV strongly vasiform with a broad band of sensoria arranged in a complete double row (Plate I, fig. 14) ... **arisaemae**
Antennal segments III and IV not vasiform and with band of sensoria narrow and sometimes reduced to a single row (Plate I, fig. 18) 15
15 (14). Terminal fringe of setae on comb plates on posterior lateral margin of abdominal tergite VI longer than length of base (Plate III, fig. 8) ... 16
Terminal fringe of setae on comb plates on posterior lateral margin of abdominal tergite VI shorter than length of base (Plate III, figs 7, 10) 18
16 (15). Basal plates on posterior margins of abdominal tergites weakly formed, short and broad ..... **quercicola**
Basal plates on posterior margins of abdominal tergites distinct, nearly as long as terminal fringe 17
17 (16). Setae and microtrichia at lateral margins of abdominal segments sparse. Antennal segment IV bicolorous ..... **lyoniae**
Setae and microtrichia at lateral margins of abdominal segments closely spaced. Antennal segment IV brown ..... **azaleae**
18 (15). Lateral portions of abdominal tergites heavily pubescent, the lines from which the microtrichia arise closely spaced ..... **vitifloridus**
Lateral portions of abdominal tergites sparsely pubescent and reticulations with or without irregularly-spaced microtrichia 19

19 (18). Microtrichia on lateral margins of abdominal tergites absent (Plate V, fig. 3). Antennal segment III 0.077 mm. long..... **xolismae**
Microtrichia present on lateral margins of abdominal tergites (Plate V, fig. 6 Antennal segment III 0.054–0.057 mm. long..... 20
20 (19). Comb plates strongly tapered. Central patch of tergal setae on abdominal segments II-V well-developed with the bases frequently fused into weak comb plates on V (and usually on VI)..... **salicis**
Comb plates slightly tapered, as broad or broader than long on V. Central patch of tergal setae on II–V very weak, sparse and bases not fused..... **aesculi**

DISCUSSION OF SPECIES

Heterothrips aesculi Watson

(Plate I, fig. 4; II, 8; IV, 8, 15; V, 6)

H. aesculi Watson, 1915, Ent. News 26(2):50–51, Pl. II, Figs. 5–6; 1924, Univ. Fla. Agr. Exp. Sta. Bul. 168:29.

We have examined the type, allotype, various paratypes, and many specimens determined by Watson in his own and other collections. Some additions should be made to the original description as follows: pronotum lightly reticulate, the cross-striations forming loose polygons. The fusion of the bases of the lateral fringe hairs on the posterior margins of abdominal segments I–VII places this species in Group II (auct.). The fringe is shorter than the bases (or plates) which are variable, sometimes slightly over-lapping or appearing to be fused. The terminal abdominal segment is short and not sharply pointed. The sternites of the male do not have oval clear areas nor are finger-like projections present on the dorsum of abdominal segment IX. Antennal segments III and IV with a circular ring of sensoria at tip arranged in a staggered double row which may be reduced to a single row dorsally. Forewings with a basal band or hyaline area.

Hood (1939) compared *aesculi* with *vernus* and *lasquerellae*, neither of which we have seen. The greatest difficulty encountered in making a positive identification of *aesculi* is that many specimens also taken from the flowers of *Aesculus pavia* and *Azalea* spp. have been misidentified apparently as *azaleae* and vice versa. Our concept of *azaleae* is that it is much more heavily pubescent on the lateral margins of the abdominal tergites, and the sternites and pronotum are much more heavily setose. We have been unable to see authentically determined specimens of *azaleae*.

The concept of host specificity breaks down in this species since *aesculi* has been collected from *Azalea indica*, *nudiflora*, *formosum*, and *canescens*, *Aesculus pavia*, and Judas tree.

It is now known from Illinois, Florida, South Carolina, and Alabama. Watson (1924) reported it from Maryland, but we have been unable to verify this northern record. All collections have been made in March, April, and May in blossoms.

We have made many thrips collections on the Pacific coast from the blossoms of *Aesculus californica* and western azaleas (*Rhododendron* spp.) and failed to find *Heterothrips*. We conclude, therefore, that this species is limited to the south Atlantic states. It is possible that in north Atlantic and north central states *azaleae* replaces *aesculi*.

Heterothrips analis Hood

H. analis Hood, 1915, Insec. Insc. Mens. 3(1–4):9–10, Pl. I, Figs. 4–5.

This species apparently has been collected only once since it was described. J. C. Crawford collected a series of what appear to be this species from wild rose on Cape May, N. J., May 12, 1937. We have examined fourteen specimens from this collection and, when compared with the original description, they exhibit some minor variations. The New Jersey specimens have the fringe on the posterior margin of the tergum of abdominal segment VI incomplete. The color variation within the series makes it difficult to separate this species from *vitis* on the basis of the coloration of the middle and hind tibiae. The antennae are colored as in "*tiliae*". The reticulations on the pronotum are more widely spaced than in paratypes of *vitis* studied. The long third antennal segment of *analis* is distinctive. The males have the finger-like projections on the dorsum of abdominal segment IX.

Until this rather restricted species is more widely collected we cannot further define its status.

The type locality is Plum Point, Maryland, and the types were taken in wild rose flowers on June 21, 1914.

Heterothrips arisaemae Hood

(Plate I, fig. 14; II, 16; IV, 13; V, 5)

H. arisaemae Hood, 1908, Bul. Ill. State Lab. Nat. Hist. 8: 361–363, Fig. 1a,b; 1925, Bul. Brooklyn Ent. Soc. 20(3):126–127, Pl. I, Fig. 1.

This distinctive species, the genotype, is readily separated from all other *Heterothrips* by its pronounced vasiform third and fourth antennal segments. It appears to be narrowly restricted to the flowers of the jack-in-the-pulpit and green dragon, *Arisaema triphyllum* and *dracontium*. Other host records are no doubt misidentifications or are accidental collections. To our knowledge this is the only species in the genus that has brachypterous males, and additional evidence that the organism is closely associated with its unique host plant. Mention also should be made of the fact that the ring of sensoria on the dorsum of antennal segments III and IV is incomplete in the male.

We have examined a number of paratypes and verified the identification of specimens from Florida, New Jersey, Tennessee, New York, Illinois, and Iowa. Watson (1924) and Hood (1925) also recorded this thrips from Virginia and Maryland. *Arisaema* does not occur

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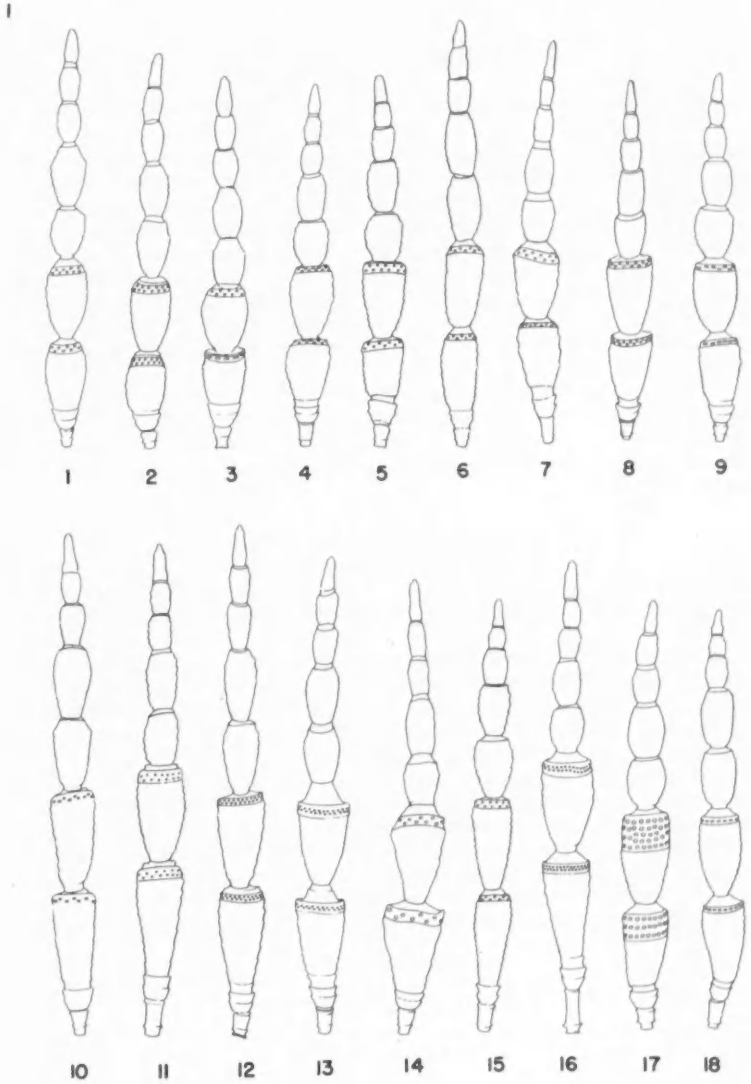
EXPLANATION OF PLATE I.

Heterothrips, antenna, segments III–IX. Fig. 1. *H. auranticornis*, type female. Fig. 2. *H. gillettei*, paratype female. Fig. 3. *H. cuernavacae*, type female. Fig. 4. *H. aesculi*, type female. Fig. 5. *H. quercicola*, paratype female. Fig. 6. *H. vitis*, paratype female. Fig. 7. *H. lyoniae*, paratype female. Fig. 8. *H. xolismae*, female. Fig. 9. *H. salicis*, female, compared with paratypes. Fig. 10. *H. mexicanus*, type female. Fig. 11. *H. limbatus*, female. Fig. 12. *H. pectinifer*, female. Fig. 13. *H. azaleae*, female. Fig. 14. *H. arisaemae* female. Fig. 15. *H. prosopidis*, paratype female. Fig. 16. *H. decacornis*, "co-type" female. Fig. 17. *H. watsoni*, type female. Fig. 18. *H. vitifloridus*, type female. X 450.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in the far western and Pacific coast states which obviously limits the insect's distribution. Hood has noted its limited occurrence and the fact that immature forms are unknown.

Heterothrips auranticornis Watson

(Plate I, fig. 1; II, 12; III, 5)

H. auranticornis Watson, 1922, Fla. Ent. 6(3): 36–37. Moulton 1929, Bul. Brooklyn. Ent. Soc. 24:228.

The type, allotype, and seven paratype slides have been studied. We make the following modifications of the original description: A double circumpolar ring of sensoria present on antennal segments III and IV. Pronotum lightly reticulate. Forewings uniform dusky brown. Microtrichia and reticulations at sides of abodminal segments sparse. Male with large, oval clear areas on sternites of abdominal segments III–VIII which on VI is 0.059 mm. (allotype) in width. It is closest to *gillettei* but may be separated from it by the bicolorous legs and the orange pigment usually present at the tips of antennal segments III and IV.

This species appears to prefer the composite flowers of *Helenium* and *Helianthus*. It is unique in that it is collected only in late summer, August 29 to September 9. The distribution of this thrips now includes Georgia, North Carolina, South Carolina, Kansas, South Dakota, and Utah.

Heterothrips azaleae Hood

(Plate I, fig. 13; II, 13; IV, 7, 17)

H, azaleae Hood, 1916, Proc. Biol. Soc. Washington, 29:110–111, Pl. III, Fig. 1. Watson, 1924, Univ. Fla., Agr. Exp. Sta. Bul. 168:29. Bailey, 1949, Fla. Ent. 32(1):22.

Watson (1924) placed this species in synonymy with *aesculi*, but we do not believe them to be identical. The confusion appears to arise in that both species are found in azalea flowers and are difficult to separate. Based on the known records, this species is restricted to *Azalea* spp. and possibly *Rhododendron*.

The original description was based on five females only, and no specimens determined by the author have been available for study. In checking many hundreds of *Heterothrips* specimens in the collections studied we have been able to separate a few specimens which we believe to be *azaleae*. A careful comparison with the type material of *aesculi* reveals differences not possible for the authors to have appreciated since the two descriptions apparently were in press at the same

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EXPLANATION OF PLATE II.

Heterothrips, female pronotum. Fig. 1. *H. lyoniae*, paratype Fig. 2. *H. vitifloridus*, type Fig. 3. *H. prosopidis*, paratype, Fig. 4. *H. vitis*, paratype, Fig. 5. *H. quercicola*, paratype. Fig. 6. *H. xolismae*, Fig. 7. *H. salicis*, paratype. Fig. 8. *H. aesculi*, paratype. Fig. 9. *H. pectinifer*. Fig. 10. *H. mexicanus*, type, Fig. 11. *H. limbatus*, Fig. 12. *H. auranticornis*, type. Fig. 13. *H. azaleae*, topotype. Fig. 14. *H. cuernavacae*, type. Fig. 15. *H. decacornis*, "co-type". Fig. 16. *H. arisaemae*, paratype. X 270.

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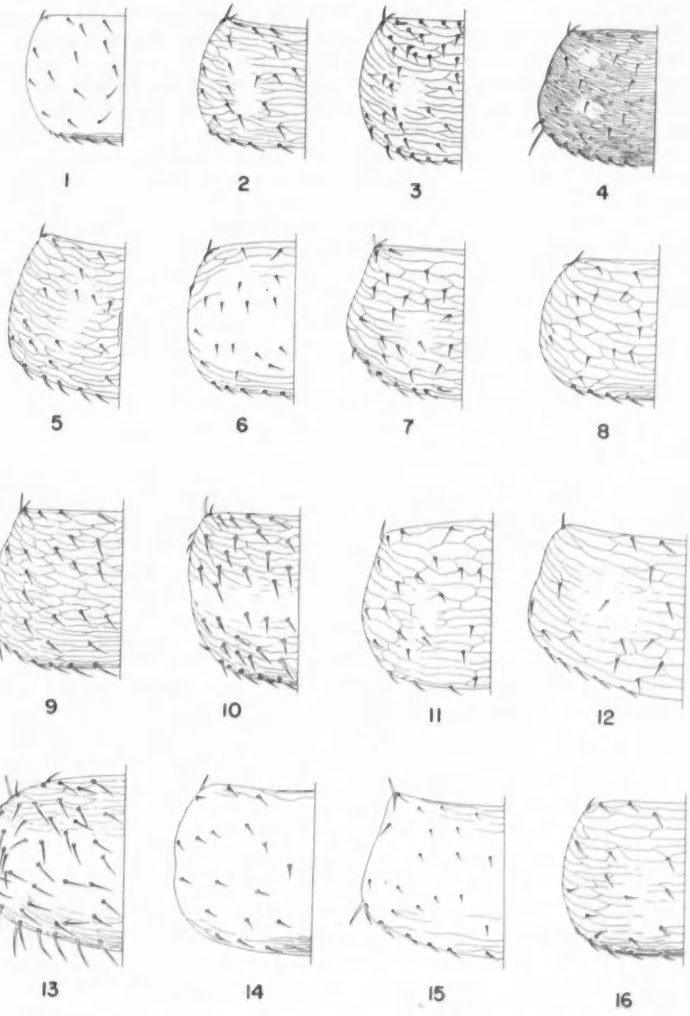

time. Both were compared with *arisaemae*, but today we utilize different and newer combinations of characters to separate the additional species since described.

It is noted that the pronotum of *azaleae* is more heavily setose as is also the venter of the abdominal segments and thorax. As predicted by Hood, the males are readily told apart by the presence of the clear, oval areas on the sternites of abdominal segments III–VIII in *azaleae;* the male of *aesculi* lacks these structures. The glandular areas in the Illinois specimens of *azaleae* are smaller than those seen from the Atlantic coast. Antennal segment IV is brown.

Upon future direct comparison of the types of both species, if our concept of *azaleae* as here illustrated proves incorrect, this species should be described as new.

The presently known distribution of *azaleae* includes Maryland, West Virginia, South Carolina, Florida, New York, and Illinois. Its seasonal appearance is somewhat later than those species inhabiting early spring flowering plants.

Heterothrips cuernavacae Watson

(Plate I, fig. 3; II, 14; III, 4; IV, 1)

H. cuernavacae Watson, 1939, Fla. Ent. 22(2):17–18, Figs. 1–2.

This Mexican species is in Group I (auct.) and is most closely related to *auranticornis* and *gillettei*. It is known only from the original collection made at Cuernavaca, Mexico, August 22, 1938, from an unknown composite. Of the three females and eight males on which the species was based, we have seen the type and six paratypes. The type specimen appears to be teneral and therefore the coloration given in the original description should be discounted somewhat. A number of additional characters should become a matter of record to make future identifications easier:

Antennae nearly all uniform brown, basal portion of segment III yellowish brown, segments III and usually IV with single dorsal row of sensoria. Pronotum with reticulation almost entirely absent. Forewings and legs brown. Fringe of setae on posterior margin of abdominal segments not strong, not fused into plates at base and incomplete on segments VI and VIII of the male. Tip of abdomen of female short. Male with large clear, oval areas on sternites III–VIII (about 0.064 mm. wide on segment VI) as in *auranticornis*.

Heterothrips decacornis D. L. Crawford

(Plate I, fig. 16; II, 15; III, 3; IV, 4)

H. decacornis D. L. Crawford, 1909, Pomona College Jour. Ent. 1(4):110–114, Fig. 50, A-I. Bailey, 1944, Pan-Pacif. Ent. 20(3):84–86, Plate I, Figs. 4, 5, 7.

This species is unique in several respects: antennal segment III is extremely long (0.090 mm.), the pronotum has strong curved setae at the outer angles, the cheeks are projected, and the bases of the comb plates on the posterior margins of the abdominal segments completely fused. The illustrations of the describer were not sufficiently accurate to supply the details needed today to separate it from the many known species. Crawford was misled in believing the third antennal segment

to be two segments and thus erroneously considered the antennae to be ten-segmented. With the exception of the third segment, which is light yellowish brown, the antennae are dark brown. The fringe of setae on the posterior margin of abdominal segment I is enlarged and fused to form a heavy comb similar to ctenidia. The male has large oval areas (about 0.065 mm. wide on segment VI) present on abdominal sternites III to VIII. No thorns or claspers present. Antennal segments III and IV have a complete double row of sensoria at the tip. The labial palpi are two-segmented. The terminal abdominal segment is much shorter than *pectinifer*. This species with its reticulations and spinose condition reminds one of the tropical heliothripoids.

In the Watson collection are three slides of this species, four females in all, from Camp Torreya, Liberty County, Florida. The collection was made by L. W. Ziegler on 4–12–30 from holly blossoms. The type series, of which we have seen 24 specimens, was taken at Guadalajara and Oxaca, Mexico, 8–15–1910, from a "low native tree, with small yellow flowers, common in the barrancas . . . ; also a shrub belonging to the family Malpighiaceae." Further collecting in the southwest should extend the distribution of this species.

Heterothrips gillettei Moulton

(Plate I, fig. 2; III, 1)

H. gillettei Moulton, 1929, Bul. Brooklyn Ent. Soc. 24(4):228–229.

This species is rare in that it is known only from the original collection which was made at Ft. Collins, Colorado in July. We have collected in this locality several times but have never been able to find it. The two hosts given by the describer, rat-tail weed and yellow sweet clover, appear to be of little value in narrowing down the host range.

It belongs in Group I and is most closely related to *auranticornis* and *cuernavacae*. From the former it may be separated by the uniformly brown legs and nearly all brown antennae. From the latter it may be distinguished by the complete double row of sensoria on antennal segments III and IV; *cuernavacae* has them reduced to a single row dorsally. The male of *gillettei* is readily separated by the lack of glandular areas on the abdominal sternites and lack of projections on the tergites. In this respect only it is related to *aesculi*. We have examined the type material in the Moulton collection and consider this a valid species.

Heterothrips lasquerellae Hood

H. lasquerellae Hood, 1939, Rev. de Ent. 10(3):554–556.

To our knowledge only the original collection of 34 specimens of this species from Texas (Brownsville and Driscoll, March, 1939) has been made. Both sexes were found in the flowers of *Lasquerella gracilis*. The describer apparently has retained all of the specimens. Since this species and *vernus* were not illustrated, and we have not seen specimens, we find it difficult to separate *lasquerellae* from *prosopidis*. The concept of different authors on what is meant by a "cross-striate" pronotum appears to vary. A comparison of the figures in Plate II

indicates the lack of distinctness of this character. And, when one considers also color variation, as well as variation in the length of the antennal segments and the abdominal glandular areas, there is reason to believe that ***prosopidis*** (also from Brownsville, Texas) may be synonymous with *lasquerellae*.

Heterothrips limbatus Hood

(Plate I, fig. 11; II, 11; IV, 11; V, 8)

H. limbatus Hood, 1925, Bul. Brooklyn Ent. Soc. 20(3):124–126, Pl. IV, Figs. 2–3; 1936, Journ. New York Ent. Soc. 44:82.

This distinct species appears to be restricted to northeastern North America and has some latitude in its host selection. We have the following new records: two males from E. Wareham, Mass., June 6, 1917, on *Vaccinium corymbosum* by H. J. Franklin, and one female from Sherborn, Mass., on May 30, 1928, sweeping, by C. A. Frost. These specimens were determined by Moulton. We have taken at Middleboro, Mass., 14 females and one male in dogwood flowers (*Cornus* sp.) on June 8, 1946. The original collection was from Macedon, N. Y., in flowers of *Prunus* in June.

As Hood pointed out, the comb is entire on the posterior dorsal margin of abdominal segments II–V (Plate V, fig. 8). This character sets it apart from all others. The male glandular areas on the sternites are variable in size.

Heterothrips lyoniae Hood

(Plate I, fig. 7; II, 1; III, 2)

H. lyoniae Hood, 1916, Proc. Biol. Soc. Washington, 29:112–113, Pl. III, Fig. 2; 1936, Jour. New York Ent. Soc. 44:82.

As more species have been described it has become increasingly difficult to place *lyoniae*. The degree of reticulation on the pronotum appears to be variable as does the length of the terminal fringe on the comb plates. The small number of specimens (11) available for study and the lack of any unique characters makes this species difficult to clearly set apart. The original description did not state that the male has the clear, oval areas on the abdominal sternites. These are present on segments III–VIII and only 0.019 mm. wide on segment VI.

We have seen one paratype taken at Brown's Mills, N. J. from *Lyonia mariana*, June 3, 1915. In addition we have seen what we believe to be this species from three localities in Florida, and one in Maryland. The specimens taken in January and March were from dead leaves, pine needles, and grass, which might indicate them to be teneral. The collections were from azalea flowers in Florida and

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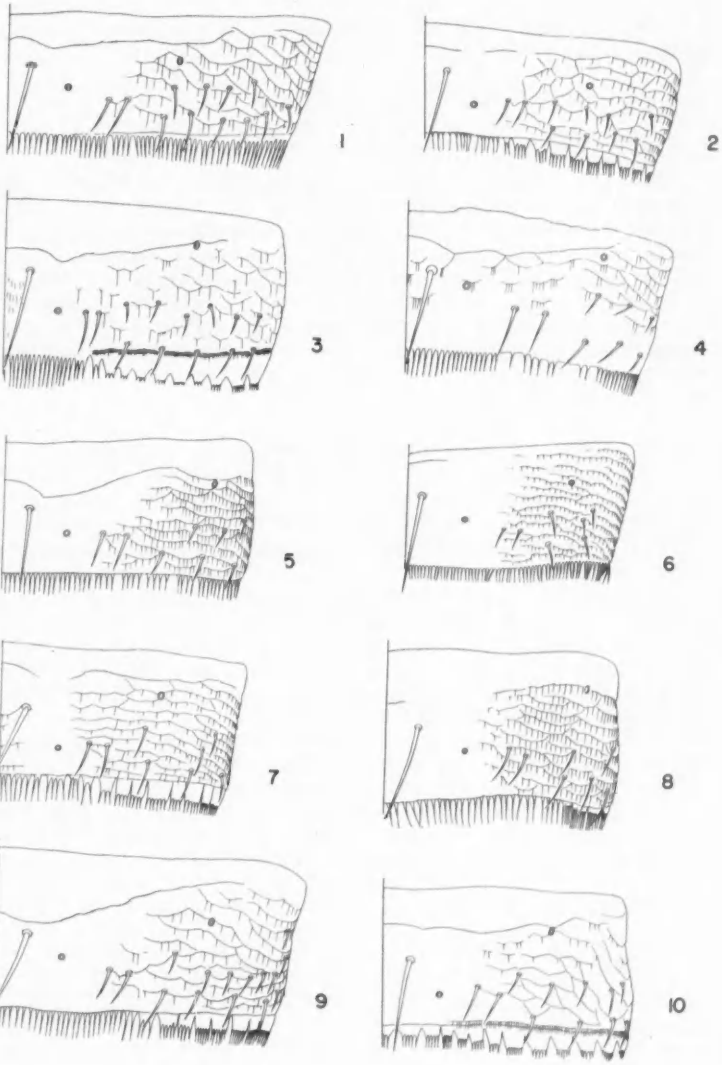
EXPLANATION OF PLATE III.

Heterothrips, female abdominal tergite VI. Fig. 1. *H. gillettei*, paratype. Fig. 2. *H. lyoniae*, paratype. Fig. 3. *H. decacornis*, "co-type". Fig. 4. *H. cuernavacae*, type. Fig. 5. *H. auranticornis*, type. Fig. 6. *H. vitis*, paratype. Fig. 7. *H. vitifloridus*, type. Fig. 8. *H. quercicola*, paratype. Fig. 9. *H. pectinifer*. Fig. 10. *H. salicis*, paratype. X 450.

III



New York. One collection seen from Maryland was collected on flowers of *Lyonia*. In describing *xolismae* twenty years later, Hood states *lyoniae* is very common in flowers of *Lyonia* which indicates the describer has made additional collections. Apparently it is restricted to the eastern seaboard.

Heterothrips mexicanus Watson

(Plate I, fig. 10; II, 10; IV, 10, 16)

H. mexicanus Watson, 1924, Fla. Agr. Exp. Sta. Bul. 168:75–76.

The describer properly compared *mexicanus* with its closest relative, *pectinifer*, but omitted mention of sufficient details of the chaetotaxy to accurately separate the two species. Considering some expected variation in color and length of antennal segments, *mexicanus* is most readily told apart by the more heavily setose pronotum. The comb plates and the arrangement of the setae on the sternites vary between the species as noted in the accompanying illustrations. We have seen 48 specimens of the type series. To our knowledge it is not known as yet from southwestern U. S. although the host plants, *Sphaeralcea* and *Sida*, are distributed widely.

Heterothrips pectinifer Hood

(Plate I, fig. 12; II, 9; III, 9; IV, 2; V, 4)

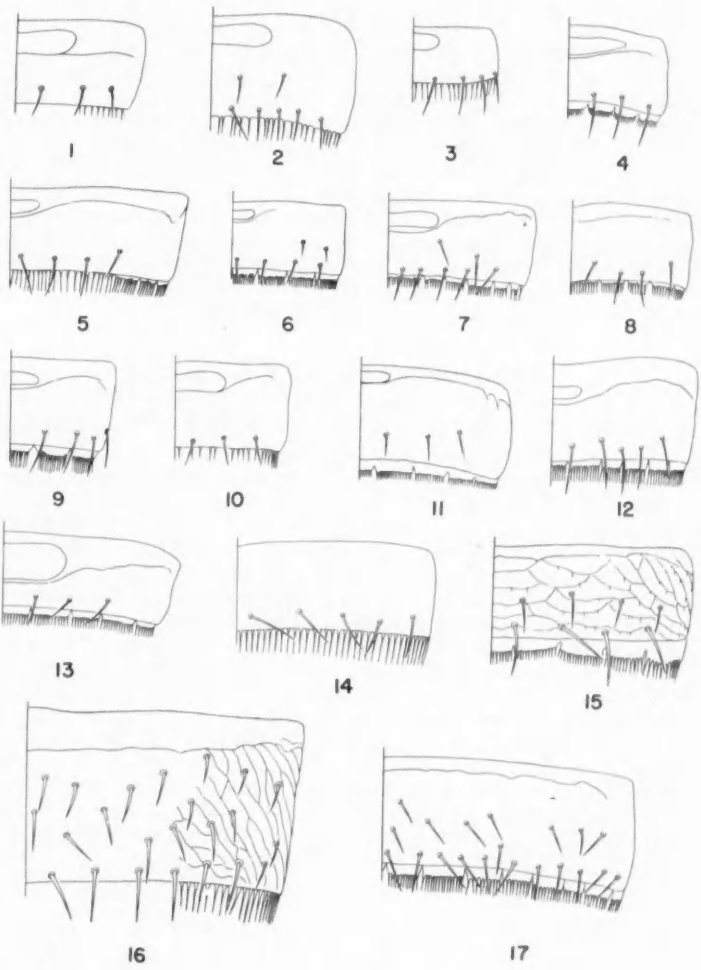
H. pectinifer Hood, 1915, Insec. Insc. Mens. 3(1–4):5–7, Pl. I, Fig. 7. Watson, 1924, Univ. Fla. Agr. Exp. Sta. Bul. 168:76.

This thrips is common and widespread in the southwestern arid areas. Its hosts are in the group of malvaceous plants known by various names as desert malva, apricot mallow, alkali mallow, Indian geranium, etc. These plants usually bloom in April, May and June. The dark brown, almost black adults are found running about rapidly within the flowers. In a long series examined from the U. S. National Museum we note one collection made in August from "native flowers" at Presidio, Texas. This late appearance may indicate a second brood or a late blooming host species in this area. One specimen has been collected on *Cleome lutea* in Utah. In addition to the original record from Tempe, Arizona, we have new records to report from Grand Canyon (South Rim) and Oak Creek Canyon. This species is known also for the first time from Castle Cliff, Marysville, Desert, Glendale, Colton, Enterprise, and Beryl, Utah. From California,

EXPLANATION OF PLATE IV

Heterothrips, abdominal sternites. Fig. 1. *H. cuernavacae*, paratype male, sternite VII. Fig. 2. *H. pectinifer*, male, VI. Fig. 3. *H. watsoni*, male, VIII. Fig. 4. *H. decacornis*, "co-type" male, VIII. Fig. 5. *H. quercicola*, paratype male, VI. Fig. 6. *H. prosopidis*, paratype male, VI. Fig. 7. *H. azaleae*, male, VI. Fig. 8. *H. aesculi*, type male, VII. Fig. 9. *H. salicis*, paratype male, VIII. Fig. 10. *H. mexicanus*, type male, VI. Fig. 11. *H. limbatus*, male, VI. Fig. 12. *H. vitifloridus*, paratype male, VI. Fig. 13. *H. arisaemae*, male, VII, slightly distorted. Fig. 14. *H. watsoni*, paratype female, VI. Fig. 15. *H. aesculi*, type female, VI. Fig. 16. *H. mexicanus*, paratype female, VI. Fig. 17. *H. azaleae*, female, VI. X 450.

IV



various entomologists have collected this thrips from Aguanga, Boron, Idyllwild, Mazurka Canyon, Palm Springs, and near Borego Springs in the desert areas. In the future undoubtedly it will be found in Nevada, New Mexico, and other adjacent areas where the hosts occur.

From its two nearest relatives, *mexicanus* and *prosopidis*, this desert species is separated by means of the long, straight-sided distinct comb plates, the long terminal abdominal segment and the long, slender terminal antennal segments.

The male of *pectinifer* has not previously been described. It may be characterized briefly as follows: Similar to but smaller than female with the usual male characters typical of the genus. Macropterous, with pale area near base of fore wing. Dorsum of abdominal segments without processes but with oval pale areas on sternites III–VIII, which area on segment VI is about 0.050 mm. wide. The comb plates are less well-developed than in the female and the posterior margins of the abdominal tergites usually with a complete fringe on segments II–VIII. The lengths of the antennal segments of a typical specimen (in mm.) are: I, 0.019, II, 0.033, III, 0.067, IV, 0.035, V, 0.035, VI, 0.037, VII, 0.028, VIII, 0.023, IX, 0.021.

Heterothrips prosopidis J. C. Crawford

(Plate I, fig. 15; II, 3; IV, 6; V, 7)

H. prosopidis J. C. Cwfd., 1943, Proc. Ent. Soc. Washington, 45(4):93–94.

This species was discussed briefly above and compared with *lasquerellae*. It has the same general appearance as *pectinifer* and also is found in the western arid areas. It can be distinguished from the species found in malva flowers by the short terminal abdominal segment and the overlapping and apparent lateral fusion of the bases of the comb plates. Sternites of male abdomen with clear areas variable, oval to round, often increasing in size posteriorly. Posterior fringe on the tergites regularly grouped into plates at base, 8–10 setae in a group. We have examined five female and two male paratypes.

The distribution of this species is now extended to California where it was found abundantly in flowers of *Prosopis* sp., June 1, 1952, 10 miles west of Randsburg in the Mojave Desert by the writer and at Bonsall (San Diego County), June 7, 1949, by R. M. Bohart. In addition to the original finding at Brownsville, Texas, we have examined twelve slides from Presidio, Texas, taken on April 7, 1944, by J. H. Russell on *Prosopis* sp. In the Moulton collection are found four specimens from Phoenix, Arizona. This thrips undoubtedly oc-

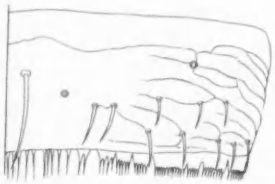
EXPLANATION OF PLATE V.

Heterothrips. FIG. 1. *H. vitis*, male, apex of abdomen, dorsal aspect. FIG. 2. *H. quercicola*, paratype male, abdominal segment X, dorsal aspect. FIG. 3. *H. xolismae*, female, abdominal tergite VI. FIG. 4. *H. pectinifer*, female, abdominal segment X, dorsal aspect. FIG. 5. *H. arisaemae*, paratype female, abdominal tergite VI. FIG. 6. *H. aesculi*, "co-type" female, abdominal tergite VI. FIG. 7. *H. prosopidis*, paratype female, abdominal tergite VI. FIG. 8. *H. limbatus*, female, abdominal tergite III. X 450.

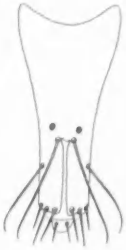
V

1

2



3



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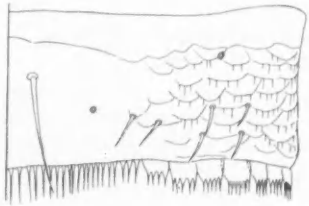

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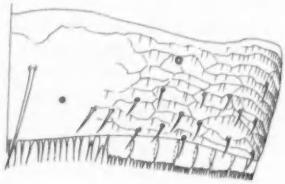
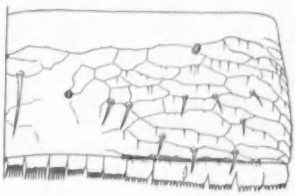
6



7



8

curs continuously between these widely separated localities and is very restricted locally to the specific host's blooming period.

Heterothrips quercicola J. C. Crawford

(Plate I, fig. 5; II, 5; III, 8; IV, 5; V, 2)

H. quercicola J. C. Crawford, 1942, Proc. Ent. Soc. Washington, 44(7):140–141.

This species reminds one of *cuernavacae* as the antennae, legs, wings, and head are short and the color dark brown. The sensoria on the dorsum of antennal segments III and IV are often reduced to a single row dorsally as in *cuernavacae*. In this latter species, a member of Group I, the bases of the posterior fringe on the lateral abdominal segments are not fused into plates while in *quercicola* they are weakly so, showing a progression in this character. Noting that *cuernavacae* was described from a small number of specimens and, that the size of the glandular areas on the male sternites varies in some species, a reevaluation of *quercicola* should be made when a greater series of the Mexican species becomes available. Another point should be made here and that is, when descriptions of *Heterothrips* are made, the same abdominal segment should always be illustrated and described in detail as the comb plates vary somewhat on the different segments.

We have seen 12 paratypes of this species and have new records from Raleigh, North Carolina, Clemson College, South Carolina, and several localities in Florida. The specimens were collected January to April and from widely different hosts as poplar, peach *Ranunculus*, maple, oak, pine, Spanish moss, saw palmetto, and a fungus.

Heterothrips salicis Shull

(Plate I, fig. 9; II, 7; III, 10; IV, 9)

H. salicis Shull, 1909, Ent. News 20(5):220–221, Fig. 1.

The original description is in need of refinement and additions. We advance the following: Antennal segments III and IV each with a circumpolar ring of sensoria which, as in *quercicola*, is reduced to a single row dorsally and sometimes even incomplete. Reticulations on pronotum very distinct, not parallel and forming polygons, setae moderately heavy. Posterior margins of abdominal segments formed laterally into tapered comb plates, distinct, and with very short setae at tip (Plate III, fig. 10). This species approaches *limbatus* in that the setae are fused into short plates at their bases on the entire central dorsum of segments VI and VII only. The other segments have scattered, incomplete central clusters on segments II to V some of which are weakly fused. This is one of the two known species in which the comb plates are strongly tapered and with the bases fused. In *decacornis* the bases are completely fused for more than half their length. The reticulations at the margins of the abdominal segments are not closely parallel and the microtrichia are sparsely placed. In general appearance it is similar to what we understand as *xolismae* and *aesculi*, with the characters of separation defined above. The male of *salicis* has the oval areas on sternites V–VIII. We have studied 18 females and 3 male paratypes from the Shull collection.

To our knowledge it is found only in willow catkins. The original collection from Huron County, Michigan, July, 1908, was from catkins of *Salix fluviatilis*. The collections from California (Alturas and Markleeville, 4400 and 5500 ft.) constitute a very great extension of its distribution. These new collections were made in the Sierras from willow catkins (probably *Salix argophylla*) in May, June, and July. Extensive collecting on willows in the coastal and great valley areas of California has not yielded any *Heterothrips*. Ross and Stannard have collected both sexes from *Salix interior* at Clinton, Illinois, in May.

Heterothrips vernus Hood

H. vernus Hood. 1939, Rev. de Ent. 10(3):552–554.

Not having seen this species, it is difficult to separate it from *quercicola*. Both species have the terminal fringe on the comb plates much longer than the base, the antennal segments are about the same length, the male glandular areas are small and present on sterna IV–VIII, and the pronotal sculpturing is closely similar. Furthermore, *vernus* was collected "from a great variety of new spring vegetation" including live oak. One might feel that, on the basis of the widely separated type localities of the two, Texas and New York, they should be distinct, but noting how we are now extending the known distribution of *auranticornis* and *salicis* it is quite conceivable they are the same. A comparison of the type series of the Crawford and Hood species should readily establish the validity of *quercicola*.

Hood described *vernus* from sixteen specimens collected in February and March at Francitas and Palacios, Texas.

Heterothrips vitifloridus, Bailey and Cott, n. sp.

(Plate I, fig. 18; II, 2; III, 7; IV, 12)

Female (macropterous). Length about 1.1 mm. Color dark brown, fore femora yellow at tip, all tarsi yellow; middle and hind tibiae light brown at tip; antennal segments I, II and V–IX brown, III yellow, IV yellow, density of yellowish brown color increasing in apical third of III, fore wings brown, with pale area in basal fourth.

Head wider than long; shape, eyes, ocelli, and reticulations normal. Antennal segment III long and slender, IV shorter than III; narrow ring of sensoria on III and IV reduced to a single row of oval, elongated areas on dorsum similar to *salicis*. Length of segments in mm.: III, 0.067, IV, 0.041, V, 0.032, VI, 0.033, VII, 0.016, VIII, 0.011, IX, 0.012. Maxillary palpi 3-segmented; labial palpi 2-segmented.

Pronotum with small scattered setae, reticulations not heavy, loosely irregular but definitely at right angles to the body axis and largely parallel. Meso—and metanotum and wings normal. Claw present on fore tarsi.

Abdomen with following critical diagnostic characters: reticulation moderately heavy, lines at sides of tergites parallel and regularly set with microtrichia; segments I–V with isolated patch of small setae in center of posterior margins. Posterior fringe of setae (laterally) on II–VII fused at bases into comb plates, these sometimes overlapping at bases and somewhat wider than long; on segment V the terminal

fringe of setae on a fully formed individual comb plate is less than the length of the plate itself, fringe complete on segments VI–VIII; terminal segment short, other characteristics normal; sternites with posterior fringe fused into broad plates with 9–12 setae each.

Male (macropterous). Similar to female but smaller as is normal in the genus. Heavily chitinized processes on dorsum of abdominal tegite IX absent; glandular areas on sternites IV–VIII small, variable in size and shape, frequently a minute circular one present on III, on segment VI of the allotype this area is 0.024 mm. wide.

Described from more than 100 specimens collected by S. F. Bailey from wild grape flowers (*Vitis californica*) from the following California localities: Indian Flat, Mariposa County, VI–8–38, Mix Canyon and Winters, Solano County, IV–30–39, Penryn, Placer County, V–19–39, Grizzly Springs, Lake County, V–14–47, and Ingot, Shasta County, V–23–49. The type locality is Winters and the holotype and allotype are in the senior author's collection.

Heterothrips vitis Hood

(Plate I, fig. 6; II, 4; III, 6; V, 1)

H.vitis Hood, 1916, Ent. News 27:106–108.
H.tiliae Watson, 1920, Fla. Ent. 4(2):29–30. New synonymy.

The species *analis* and *vitis* (including *tiliae*) constitute a small group set apart from the other members of the genus by lack of comb plates, finely cross-striate pronotum, and the bicolorous tibiae. Since color is often unreliable, the most distinctive character with which to separate the two is the long third antennal segment of *analis*. We have examined all the specimens of *tiliae* and *vitis* in the Watson collection and cannot separate them. The only difference noted is the light colored fourth antennal segment (see also *analis*) in *tiliae*. Until such time as the types of *analis* can be studied, *tiliae* must be considered synonymous with *vitis*.

The species *vitis* was described from Maryland, D. C., and Virginia. Our new records include collections from Colorado, Illinois, Michigan, and Tennessee. Many specimens from Florida locations are in the Watson collection. The majority of specimens examined are from wild grape flowers but other hosts are *Juncus*, mountain laurel, *Ceanothus*, *Viburnum*, herbs, grass, willow, *Smilax*, and poison ivy.

We have seen three paratypes and other specimens determined by Hood.

Heterothrips watsoni Bailey and Cott, n. sp.

(Plate I, 17; IV, 3, 14)

Female (macropterous): Length about 1.06 mm. Color brown, fore femora yellowish brown in distal half, all tarsi yellow; middle and hind tibiae brown, lighter at extremities; antennal segments I, II, V–IX brown, III yellow, IV bicolorous, basal five-eighths brown, remainder yellow; fore wings brown with pale area in basal fourth.

Head wider than long; width behind eyes 0.144 mm.; shape, eyes, ocelli, and reticulations normal. Antenna short; segment III vasiform, IV with sides parallel in distal portion, V–IX compact but

articulated; III and IV each with a broad band of sensoria arranged in *three rows* which encircle segments in distal portion, two simple cones on both V and VI. Length of segments in mm.: I, 0.019, II, 0.031, III, 0.057, IV, 0.041, V, 0.020, VI, 0.023, VII, 0.013, VIII, 0.011, IX, 0.014. Maxillary palpi 3-segmented; labial palpi 2-segmented.

Pronotum with small scattered setae, reticulations not heavy and arranged in a cross-striate manner over entire surface, some of the striations joining irregularly; width 0.179 mm., length 0.124 mm. Meso- and metanotum and wings normal. Claw on fore tarsi.

Abdomen with lateral reticulations dense and parallel with regularly spaced microtrichia; fringe of setae on posterior margins not joined at bases to form plates; fringe complete dorsally on VI–VIII only; complete fringe on sternites made up of long slender setae; terminal segment short.

Male (macropterous): Similar to female but smaller. Glandular areas present on sternites of abdominal segments V–VIII, that on V the smallest, on VI this area is 0.017 x 0.010 mm. Processes on dorsum of segment IX absent.

Described from 31 females and 4 males. Four paratype slides, making up 30 females and 3 males, are from Rio Frio, Quebrada, Rodriquez, Colombia, collected 3–15–25 by F. W. Walker (no host data given). The holotype female and allotype are from the Watson collection, "separated in Berlese funnel from dead petioles of *Washingtonia robusta*, 2–5–26, Gainesville, Fla., D. M. Bates, coll". The specimens were recognized as new by Watson and he had given the two lots different manuscript names. To our knowledge the only specimens distributed were to the U. S. National Museum. Of these four specimens, two (the types) are numbered 40906. We are naming this species after Professor Watson who recognized it as new. The allotype and all but one paratype slide are deposited in the U. S. National Museum.

This *Heterothrips* is easily separated from all other known species by the broad band of sensoria on antennal segment III and IV. In general appearance it is similar to *aesculi* but is a member of Group I in which *analis* and *vitis* both have the pronotum heavily cross-striated.

Heterothrips xolismae Hood

(Plate I, fig. 8; II, 6; V, 3)

H. xolismae Hood, 1936, Jour. New York, Ent. Soc. 44:81–83.

This eastern species is very close to *salicis*. The possibility of expanding its description is limited by such a small number of known specimens (seven in the original collection). We have seen six female specimens collected and identified as this species by J. C. Crawford at Vienna, Va., from *Xolisma*, June 18, 1941. In these individuals we note the reticulations on the pronotum and abdomen are much less distinct than in *salicis* and the microtrichia at the lateral margins are almost entirely absent. The comb plates are broader and do not taper strongly as in *salicis*. This is the only species discussed in this paper in which the male is unknown.

List of Known HETEROTHRIPS

aesculi Watson, 1915
analis Hood, 1915
arisaemae Hood, 1908
auranticornis Watson, 1922
azaleae Hood, 1916
borinquen Hood, 1915
brasiliensis Moulton, 1932
condei Moulton, 1932
cuernavacae Watson, 1939
fiecacornis D. L. Crawford, 1909
dmbriatus Hood, 1934
flavicornis Hood, 1915
flavicruris Hood, 1934
flavitibia Moulton, 1932
gillettei Moulton, 1929
lasquerellae Hood, 1939
limbatus Hood, 1925
lyoniae Hood, 1916
mexicanus Watson, 1924
miconiae Hood, 1937
minor Hood, 1915
moreirai Moulton, 1932
nudus Moulton, 1932
ornatus Hood, 1938
pastranai Tapia, 1952
pectinifer Hood, 1915
peruvianus Hood, 1936
pilosus (Buffa), 1908 (from *Phyllothrips* Buffa, nec. Hood)
prosopidis J. C. Crawford, 1943
pubescens Hood, 1934
quercicola J. C. Crawford, 1942
salicis Shull, 1909
sericatus Hood, 1913
spinosus Moulton, 1932
striatus Moulton, 1932
tiliae Watson, 1920=*vitus* Hood.
varitibia Moulton, 1932
vernus Hood, 1939
vitifloridus Bailey and Cott, n. sp.
vitis Hood, 1916
watsoni Bailey and Cott, n. sp.
xolismae Hood, 1936

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CALIFORNIA VECTOR VIEWS. To date four numbers of this publication have appeared, at monthly intervals, the first being dated July, 1954. So far, it has been issued as a four-page bulletin, prepared by a photographic offset method, with one leading article to each issue, and the remainder devoted to brief articles, notes, news items, a short question-and-answer department, and some book notices. The leading articles, so far, have been: "Choosing a refuse disposal method", by Ralph J. Black; Oakland's vector control program", by Alfred N. Rea; "Arthropod-borne encephalititis in California", by Wm. Allen Longshore; and "Use of pesticides by public agencies", by Allen B. Lemmon. The editor is Arthur C. Smith, Bureau of Vector Control, State Department of Public Health, Berkeley.

THE FOOD REQUIREMENTS OF THE ADULT FEMALE BLOW-FLY, PHORMIA REGINA (MEIGEN), IN RELATION TO OVARIAN DEVELOPMENT

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It is well known that the ovaries of flies are totally undeveloped at emergence and that female flies require a diet consisting of water, sugar and protein; water and sugar for maintenence of life and protein for egg development. Glaser (1923) found that houseflies could produce a few eggs on sugar and water alone, but that the combination of sugar and bouillon produced many more eggs. Mackerras (1933), Evans (1935), Dorman, Hale and Hoskins (1938) and Hobson (1938) demonstrated that *Lucilia sericata* did not develop their ovaries in the absence of a protein. According to Dorman *et al.*, flies kept for 6 weeks on sugar and water, produced eggs when given fish. Casein with butter and yeast, soluble casein, blood albumen and Lemco beef extract could not replace fish. The same fly, according to Hobson (1938), required for egg production a meal of meat juice which could not be replaced by milk or blood serum, but could be replaced by a combination of serum and autolysed yeast. Dean (1938), working with the apple-maggot adult, found that they laid no eggs with sugar alone, but did so on sugar and peptone.

It has therefore been clearly demonstrated that protein and sugars are required for egg production by many common flies. In none of these investigations, however, had proteins of sufficient purity been used to insure the absence of vitamins or minerals. It is the object of this paper to determine the effects of sugar, purified proteins, B-vitamins and minerals on the development of the ovaries of *Phormia regina*.

METHODS AND MATERIALS USED

The larvae of *Phormia regina* were raised on fresh beef liver and allowed to pupate in sawdust. For each test, 250 pupae were placed into a screened cage, 7½″ x 7½″ x 7½″. The emerging adults were supplied with cube sugar and water; drowning was prevented by presenting the water in closed glass vials with exposed cotton wicks. All testing was conducted in the laboratory at room temperature, which most of the time was approximately 23° C.

Experimental diets were prepared by weighing out the dry components and transferring them to a Syracuse staining dish containing 15cc of tap water. Dry cube sugar (sucrose) was added to all experiments unless otherwise designated. All diets were supplied as solutions or suspensions. Paper toweling was floated on the surface to facilitate feeding. The sources of foods used in experimental diets are listed below:

[1]In partial fulfillment of the requirements for a Master of Science degree.

FOOD	SOURCE	
Whey (milk)	Western Condensing Co.	Appleton, Wisconsin
Dried Brewers' yeast	General Biochemicals, Inc.	Chagrin Falls, Ohio
Sodium caseinate (technical)	Eastman Kodak Co.	Rochester, NewYork
Nutrose (sodium caseinate)	Pfanstiehl Chemical Co	Waukegan, Illinois
Casein (vitamin-free)	General Biochemicals, Inc.	Chagrin Falls, Ohio
Casein hydrolysate (enzymatic)	General Biochemicals, Inc.	Chagrin Falls, Ohio
Casein hydrolysate 5% (enzymatic) vitamin free	Nutritional Biochemicals Corp.	Cleveland, Ohio
Albumen (egg)	Nutritional Biochemicals, Inc.	Cleveland, Ohio
Salt mixture (Wesson's modification)	General Biochemicals, Inc.	Chagrin Falls, Ohio

Vitamins (B-Complex). The vitamin mixture was made up of the following vitamins:

VITAMINS	*MILLIGRAMS IN 10 cc. WATER*
Thiamin	5.0
Riboflavin	2.5
Nicotinic acid	10.0
Pantothenic acid	5.0
Pyridoxin	2.5
Choline chloride	100.0
Inositol	50.0
Folic acid	0.5
Biotin	0.1

The B-vitamins were then pipetted into experimental diets at the rate of 1.0 cc. in 15 cc. water. In later experiments, with individual vitamin deficiences, stock solutions were prepared with all vitamins but one.

Minerals. The salt mixture was Wesson's modification of the original Osborne-Mendal Salt Mixture, and consisted of the following components:

Minerals	*mg/gram*	*Minerals*	*mg/gram*
Potassium Phosphate	310.0	Ferric Phosphate	14.70
Calcium Carbonate	210.0	Sodium Fluoride	0.57
Tricalcium Phosphate	149.0	Copper Sulphate	0.39
Potassium Chloride	120.0	Manganous Sulphate	0.20
Sodium Chloride	105.0	Potassium Al. Sulphate	0.09
Magnesium Sulphate	90.0	Potassium Iodide	0.05

The proteins were used in 6% solutions or suspensions and the salt mixture was added in amounts of 0.1 gram per 15cc. solution.

Dialysis. Proteins were purified by means of dialysis. The materials were placed in visking casings and suspended in running water for 72 hours. The dialysates in all experiments were replaced at 24 hour intervals to reduce possible bacterial contamination.

Dissection and Measurement. Twenty female flies were removed from each experimental cage at 24 or 48 hour intervals by taking advantage of their positive phototaxis. They were immediately anesthetised with carbon dioxide or ether, and submerged in water. Dissections were made by making two lateral longitudinal incisions along the abdomen and removing the tergum, thus exposing the ovaries.

The right ovary of each fly was placed in 70% alcohol, and its length measured under a calibrated microscope.

It was found that adults on an optimal diet attain maximum ovarian development at about 72 hours; consequently experiments were terminated in most cases at 120 hours, but sometimes were continued for longer periods. At the termination of the feeding period the flies were offered liver for several hours, and if eggs were deposited, they were observed for hatching. In tabulating the results, the following symbols were used:

\+ indicates production of eggs
\++ eggs viable
\− no eggs produced

RESULTS OF FEEDING EXPERIMENTS

A. Sugars alone

In the presence of sugars alone, *Phormia* produced no eggs and for all practical purposes the ovaries remained undeveloped. Of the sugars tested, galactose, mannose, fructose, maltose and sucrose proved to maintain life for 21 days or longer, while on lactose and xylose death occured after about 13 days. These results are in substantial agreement with the survival experiments of Hassett, Dethier and Gans (1950) on *Phormia regina*, and of Haslinger (1936) and Fraenkel (1936) on *Calliphora erthyrocephala*.

B. Proteins alone

A number of different proteins, or sources of protein, were tested in the presence of water but the absence of a carbohydrate. These included brewers yeast, whey of milk alone and with the addition of yeast, egg albumen alone and with the addition of yeast, sodium caseinate (technical) and casein hydrolysate (enzymatic). In none of these cases did eggs develop. All adults died within 6 days.

C. Liver and milk

It was desirable to compare the effect of two natural diets, liver and evaporated milk, in the presence of sucrose, with respect to maturation of the ovaries. As shown in table I, milk supported egg production as well, or almost as well as liver.

TABLE I

COMPARISON BETWEEN THE NATURAL DIET (Liver) AND MILK ON OVARIAN DEVELOPMENT

DIETS Sucrose Present	LENGTH OF OVARIES IN MILLIMETERS					PRODUCTION OF EGGS	VIABILITY OF EGGS
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.		
1. Control (liver)	2.14	3.45	3.73	4.02		+	++
2. Milk (evaporated)	1.80	2.75	3.20	3.50	3.65	+	++
3. Control (sucrose)					1.32	−	

Length of ovaries at emergence 1.03 millimeters.

D. Protein plus sucrose

Of several proteins or sources of proteins tested, sodium caseinate, casein hydrolysate, egg albumen, and brewers yeast gave good egg production (Table II). Eggs developed slowly in the presence of vitamin-free casein or whey. Whey proved an inadequate diet presumably owing to its low protein content. When the concentration of whey was increased fivefold, ovaries developed normally and viable eggs were produced. The relative inefficiency of vitamin-free casein was due to its insolubility in water. When the same preparation was

TABLE II

EFFECTS OF DIFFERENT PROTEINS IN THE PRESENCE OF SUCROSE ON OVARIAN DEVELOPMENT AND EGG VIABILITY

DIETS	LENGTH OF OVARIES IN MILLIMETERS					PRODUCTION OF EGGS	VIABILITY OF EGGS
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.		
1. Control (liver)	2.05	4.12	4.37			+	++
2. Control (sucrose)					1.13	−	
3. Sodium caseinate	1.75	2.62	3.12	3.50	3.60	+	++
4. Casein hydrolysate (enzymatic)	1.70	2.50	1.87	3.05	3.12	+	++
5. Egg Albumen	1.60	2.20	2.50	2.62	2.66	+	++
6. Brewers' yeast	1.50	1.86	2.27	2.50	2.58	+	++
7. Casein (vitamin free)	1.17	1.25	1.42	1.53	1.65	−	−
8. Whey (milk)	1.07	1.10	1.23	1.30	1.37	−	

Length of ovaries at emergence 1.00 millimeters.

TABLE III

EFFECTS OF INSOLUBLE AND SOLUBLE CASEIN (VITAMIN FREE) ON THE RATE OF OVARIAN DEVELOPMENT AND VIABILITY OF EGGS

DIETS SUCROSE PRESENT	LENGTH OF OVARIES IN MILLIMETERS HOURS								PRODUCTION OF EGGS	VIABILITY OF EGGS
	24	48	72	96	120	144	168	216		
1. Control (liver)	2.60	3.56	4.53	4.85					+	++
2. Control (sucrose)								1.11	—	
3. Sodium caseinate[1]					2.94	3.34	3.70	3.81	+	++
4. Casein (vitamin free)					1.71	2.36	2.75	2.96	+	++
5. Sodium caseinate (technical)				3.41	3.55	3.80	4.05		+	++

Length of ovaries at emergence 0.91 millimeters.

[1]Casein (vitamin free) dissolved in 0.1 N sodium hydroxide.

converted to the water soluble sodium salt, it was readily utilized for egg production (Table III). Similarly casein made soluble by enzymatic hydrolysis supported egg development (Table II).

It must not be assumed that vitamin-free casein was incapable of supporting egg production. The tests of table II were terminated after 120 hours. If however they were continued to 216 hours (Table III) viable eggs were produced.

E. Protein—sugar diet plus yeast or whey

Sodium caseinate (technical) proved to induce ovarian development faster than sodium caseinate prepared from vitamin-free casein (Table III). This may have been due to the presence, in technical casein, of impurities such as vitamins or minerals. It was, therefore, considered advisable to test the effect of the addition of yeast, yeast extract and whey as good sources of B-vitamins and minerals on egg production. In all instances where the additions were made a decided improvement in the diet was noticed (Table IV). This suggested the possibility of vitamin and/or mineral requirements.

Of the additions, when used alone, yeast extract proved to be very efficient in promoting egg development, even more efficient than yeast, which may have been due to its greater solubility. Whey alone had little effect, as already reported.

Yeast extract was prepared by boiling 10 g. dried brewers yeast in 100 ml. water for 10 minutes and centrifuging. The supernatant was used.

F. B-vitamins.

Since brewers yeast, yeast extract and whey speeded up ovarian development when added to a protein, the effect of a mixture of pure B-vitamins was tested. As shown in Table V, all diets tested were somewhat improved by the addition of a mixture of 9 B-vitamins, with the exception of technical sodium caseinate which might already have contained vitamins. The improvements were, however, not striking.

G. Dialysis of technical sodium caseinate.

An attempt was then made to free sodium caseinate (technical) of diffusable contaminants by dialysis. Dialyzed sodium caseinate proved to be far inferior to the untreated material, which suggested the loss of important dietary factors. When vitamin and mineral mixtures were added to dialyzed preparations the original efficiency was restored. The addition of minerals appeared to be more important in maturation of ovaries than that of B-vitamins (Table VI).

H. Individual B-vitamin requirements.

Diets deficient in individual B-vitamins were tested. The basic diet consisted of sodium caseinate prepared from vitamin-free casein, sucrose and the mixture of nine B-vitamins. The absence of folic acid, inositol, thiamin, pantothenic acid, riboflavin, or pyridoxin from the vitamin mixture, had little or no effect on egg development. Omission of choline or nicotinic acid appeared to have a slightly detrimental effect, judging from the result at 48 hours.

TABLE IV

EFFECTS OF THE ADDITION OF BREWERS YEAST, YEAST EXTRACT AND WHEY TO VARIOUS PROTEINS ON OVARIAN DEVELOPMENT AND EGG VIABILITY

DIETS SUCROSE PRESENT	LENGTH OF OVARIES IN MILLIMETERS					PRODUCTION OF EGGS	VIABILITY OF EGGS
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.		
1. Control (liver)	3.25	4.00	4.13			+	++
2. Control (sucrose)					1.25	—	
3. Sodium caseinate (technical)	1.70	2.55	2.79	2.92	3.00	+	++
4. Sodium caseinate (technical) plus yeast extract	2.30	3.50	3.72	3.83	3.88	+	++
5. Egg albumen	1.57	2.17	2.51	2.66	2.72	+	++
6. Egg albumen plus brewers' yeast	2.12	2.99	3.25	3.37	3.40	+	++
7. Casein hydrolysate (enzymatic)	1.65	2.22	2.64	2.85	2.90	+	++
8. Yeast extract	2.20	2.77	3.13	3.45	3.52	+	++
9. Brewers' yeast	1.50	1.90	2.47	2.75	2.85	+	++
10. Whey (milk)	1.25	1.37	1.45	1.50	1.67	—	

Length of ovaries at emergence 0.95 millimeters.

TABLE V

EFFECTS OF ADDITION OF A MIXTURE OF NINE VITAMINS (B—Complex) TO VARIOUS PROTEINS ON OVARIAN DEVELOPMENT AND VIABILITY OF EGGS

DIETS SUCROSE PRESENT	LENGTH OF OVARIES IN MILLIMETERS					PRODUCTION OF EGGS	VIABILITY OF EGGS
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.		
1. Control (liver)	3.00	3.82	4.00			+	++
2. Control (sucrose)					1.30	—	
3. Casein hydrolysate (enzymatic)	2.14	2.62	2.80	2.90	2.95	+	++
4. Casein hydrolysate (enzymatic) plus vitamins	2.25	2.87	3.14	3.22	3.25	+	++
5. Egg albumen	1.57	2.03	2.37	2.72	2.87	+	++
6. Egg albumen plus vitamins	1.80	2.45	2.77	2.95	3.11	+	++
7. Sodium caseinate[1]	1.40	1.90	2.25	2.50	2.62	+	++
8. Sodium caseinate[1] plus vitamins	1.67	2.35	2.70	2.85	3.02	+	++
9. Sodium caseinate[2]	1.25	1.50	1.66	1.77	1.94	+	++
10. Sodium caseinate[2] plus vitamins	1.35	1.70	1.90	2.05	2.12	+	++
11. Vitamins (B—complex)					1.20	—	

Length of ovaries at emergence 0.97 millimeters.

[1]Sodium caseinate (technical).

[2]Sodium caseinate prepared by dissolving vitamin free casein in 0.1 N sodium hydroxide.

I. Inorganic salts.

Since the importance of additional minerals in the diet was noted in Table 6, a test was designed to show the effects of the addition of the salt mixture to various proteins on egg development. Table VII clearly indicates that in all instances where addition of salts were made the rate of ovarian development increased markedly.

A test was then devised to show which components of the salt mixture were especially necessary. Addition of potassium phosphate markedly speeded up ovarian development. The other salts tested individually, potassium chloride, magnesium sulfate, potassium carbonate, sodium chloride and tricalcium phosphate had no effect.

DISCUSSION

It has been confirmed that muscoid adults must be supplied with protein and sugar for the production of viable eggs. However, certain phases of the investigations by Glaser (1923), Dorman *et al.* (1938) and Hobson (1938) have not been substantiated in this study. For example, Glaser (1923), working with the housefly, *Musca domestica*, found a few eggs produced on a sucrose solution, whereas the blowfly, *Phormia regina*, was unable to develop its ovaries on a diet of sucrose and water alone. Dorman *et al.* (1938), in their study with *Lucilia sericata*, found certain proteins (casein, sodium caseinate, blood albumen) ineffective in supporting egg development when added to a diet of sugar and water. This finding was in contrast with that obtained with *Phormia* in which casein, sodium caseinate or egg albumen, when added to a sugar solution, supported egg development. *Phormia*, contrary to *Lucilia* (Hobson 1938) develops eggs very well on milk.

Superficially, it may appear that certain proteins or protein-containing substances are inadequate for the production of eggs. The whey of milk, for example, at first proved to be an inadequate diet owing to its low protein content. When the concentration of whey was increased fivefold ovaries developed normally and viable eggs were produced. Vitamin-free casein also appeared inadequate for the development of ovaries. However, the inefficiency of vitamin-free casein was due to its insolubility in water. When the casein was converted to a soluble form, sodium caseinate, by dissolving in 0.1 N sodium hydroxide, it was readily utilized for egg development.

As shown in the experiments outlined, ovaries matured slightly more rapidly with inclusions of B-vitamins in the basic protein-carbohydrate diet. The presences of B-vitamins, however, was not essential for the development of ovaries. The following factors must be considered in interpreting the results: (1) the absence of sterility of the flies, (2) storage of vitamins by the larvae, and (3) the purity of the proteins tested. One must not assume that the only substances entering the intestinal lumen were the experimental diets. Bacteria in the lumen, introduced during feeding, may have the power to synthesize vitamins and other necessary factors. Secondly, adults carry over with them vitamins stored during larval development. Since it is impossible to rear fly larvae in the absence of B-vitamins it is obvious the larvae must have had access to an adequate vitamin supply. The

TABLE VI

EFFECTS OF ADDITION OF VARIOUS SUBSTANCES TO DIALYZED SODIUM CASEINATE (Technical) ON OVARIAN DEVELOPMENT AND EGG VIABILITY

DIETS SUCROSE PRESENT	LENGTH OF OVARIES IN MILLIMETERS				PRODUCTION OF EGGS	VIABILITY OF EGGS
	48 hrs.	96 hrs.	144 hrs.	192 hrs.		
1. Control (liver)	3.55	3.98			+	++
2. Control (sucrose)				1.21	—	
3. Sodium caseinate[1]	2.30	3.05	3.37	3.51	+	++
4. Sodium caseinate[1] (dialyzed) plus vitamins plus salt mixture	2.17	2.96	3.35	3.45	+	++
5. Sodium caseinate[1] (dialyzed) plus salt mixture	2.13	2.78	3.10	3.19	+	++
6. Sodium caseinate[1] (dialyzed) plus vitamins	1.84	2.35	2.75	2.80	+	++
7. Sodium caseinate[1] (dialyzed)	1.66	2.10	2.32	2.50	+	++
8. Vitamins				1.35	—	

Length of ovaries at emergence 0.97 millimeters.
[1]Sodium caseinate technical.

TABLE VII

EFFECTS OF THE ADDITION OF INORGANIC SALT MIXTURE TO PROTEINS ON EGG DEVELOPMENT AND VIABILITY OF EGGS

DIETS SUCROSE PRESENT	LENGTH OF OVARIES IN MILLIMETERS					PRODUCTION OF EGGS	VIABILITY OF EGGS
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.		
1. Control (liver)	2.31	4.03	4.55	4.60		+	++
2. Control (sucrose)			1.08		1.10	—	
3. Casein hydrolysate	1.69	1.85	2.39	2.58	2.77	+	++
4. Casein hydrolysate plus salt mixture	1.96	2.72	3.39	3.58	3.85	+	++
5. Egg albumen	1.78	1.99	2.26	2.43	2.50	+	++
6. Egg albumen plus salt mixture	1.88	2.96	3.57	3.63	3.70	+	++
7. Sodium caseinate[1]	1.40	1.98	2.11	2.87	2.98	+	++
8. Sodium caseinate[1] plus salt mixture	2.00	3.02	3.40	3.56	3.60	+	++
9. Sodium caseinate[2]	1.24	1.54	2.32	2.55	2.60	+	++
10. Sodium caseinate[2] plus salt mixture	1.76	2.34	2.99	3.10	3.15	+	++

Length of ovaries at emergence 1.05 millimeters.
[1]Sodium caseinate (technical).
[2]Sodium caseinate prepared by the addition of 0.1 N Na OH to vitamin test casein.

purity of the substance tested also is of the utmost importance in determining the necessity for the B-vitamins. "Vitamin-free" casein is never entirely free of B-vitamins, and may contain sufficient quantities for egg development.

The addition of minerals (Wesson's salt modification) to the basic carbohydrate-protein diet had a far greater effect on maturation of ovaries than the B-vitamins. Potassium phosphate alone had the same effect as the complete salt mixture. Other individual minerals such as sodium chloride, potassium carbonate, magnesium sulphate, potassium chloride and tricalcium phosphate had little or no effect on the ovaries. The fact that the above mentioned minerals had no effect on egg development may have been due to the following factors: (1) the presence of minerals in the water used, since all experimental diets were dissolved in tap water, (2) an excess of sodium ions introduced in all diets where casein was made soluble by treating with 0.1 N sodium hydroxide, (3) the insolubility of tricalcium phosphate, (4) a concentration of salts in excess of the normal requirement. It is obvious that further investigations of the mineral requirements are warranted.

Even on the best synthetic diets, which included soluble casein, sucrose, B-vitamins and minerals, egg development was much slower than on liver. Obviously, important dietary factors were still absent from the prepared diets.

SUMMARY

1. The blow-fly, *Phormia regina*, on a diet of water and sugar, shows no development of ovaries.

2. Adult flies, in the absence of sugar, produce no eggs on a diet of protein or products of protein hydrolysis, and live for a maximum of 5 days at room temperature.

3. In the presence of sugar, eggs are produced with proteins or protein-containing substances such as casein, sodium caseinate, casein hydrolysate (enzymatic), egg albumen, brewers yeast, yeast extract, milk and whey.

4. Proteins or protein-containing substances must be in a soluble form to insure ovarian development.

5. *Phormia* are able to produce eggs on sodium caseinate purified by dialysis but more slowly than on the non-dialysed preparation.

6. The addition of B-vitamins and minerals to a purified protein somewhat accelerated egg development. These effects were found to be due mainly to choline, nicotinic acid and potassium phosphate.

7. Even on the best synthetic diets egg production was very much slower than on liver.

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AN INTRODUCTION TO THE STUDY OF INSECTS, by DONALD J. BORROR and DWIGHT M. DELONG. Pp. ix +1030, 623 figs. Rinehart & Co., New York. 1954. Price, $9.00.

The authors approach the study of entomology from the systematic standpoint. After three introductory chapters, in which the necessary background of insect structure and development is laid, and a chapter on "Classification, Nomenclature and Identification", the orders and most important families are treated (pp. 72–763), with keys, diagnoses, condensed discussion, some exemplification, and abundant illustration. The chapter following (pp. 764–809) covers arthropods other than insects in a similar but more restricted way, and the remaining chapters deal with economic relationships and control, collecting and preserving methods, and (a unique feature) suggestions as to activities and projects in insect study. There is a glossary and an extensive index. The book is reporduced by a new photoprint process that appears to be increasing in popularity because of its lower cost and at the same time satisfactory results. It is very well bound and in general presents a very good appearance. Mechanically, it should be quite serviceable as a textbook.

Among the excellent features of the book are the clarity and conciseness of the language, the indication of proper pronunciation of scientific names (both, in general, in an introductory section, and, in particular, in places where the names appear in the text), the wealth of well chosen illustrations, including many original drawings and photographs, and the inclusion of collecting methods at the ends of the individual chapters, as well as the more usual discussion of collecting, preserving, and museum methods in a separate chapter. Drawings were made by different individuals under the supervision of Dr. DeLong. Consequently, there is some variation in their degree of excellence; in general, however, they are very satisfactory, and some are excellent. The selection of borrowed material was well done.

Errors, both of statement and typography, appear to be few. Prior to publication, the manuscript was submitted, in part or whole, to specialists in various fields, and this has undoubtedly aided in reducing the number of errors and ambiguities. (If all authors and publishers had the foresight to do this, editors would be relieved of much of the embarrassment of having to publish unfavorable reviews). The classification will not please everyone; in some cases, including certain Homoptera, an order in which the senior author is a specialist, it might be considered ultraconservative. Some changes might have been made in the selection of reference lists in certain groups. The inclusion of well-known synonyms of certain families is a very useful one which should appease specialists who disagree with the selection of family names used by the authors.

This book fills an important void which has existed in entomological text book literature—an approach to a taxonomic treatment of the subject which is not too heavy for an introductory course on an upper-division plane, which is up-to-date, and which adopts a relatively conservative classification. It deserves serious consideration as a text for any course in introductory systematic entomology or for any general course which approaches the subject from the systematic standpoint.—M.T.J.

STUDIES ON THE LIFE HISTORY OF THREE ORIBATOID MITES WITH OBSERVATIONS ON OTHER SPECIES

(Acarina, Oribatei)[1]

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Detailed knowledge of the development of the larvae and nymphs of the oribatoid mites is virtually non-existent. Dr. Edward W. Baker, of the Entomology Research Branch, U. S. Department of Agriculture has stated (personal communication) that there is no literature available by which the immature stages of this group of mites can be identified.

To supply some of this information it was first necessary to discover a method by which oribatoid mites could be reared successfully in the laboratory so that the length of their developmental stages could be traced, and so that larvae and nymphs could be observed daily to note any changes in form and behavior that would aid in their identification. In this introductory study the collection of life-history data was limited to three species of *Galumna* in the belief that once the technique and pattern had been established, future work in this field could fill in the remaining gaps of information. Some observations on species of other genera have, however, been included.

Oribatoid mites, although once thought to be all harmless plant feeders, are now known to be of great economic importance. Sellnick (1928) believed that they play an important role in the economy of nature in that they contribute considerably to the fertility of the forest soil. Jacot (1930a), on the other hand, pointed out injurious effects which could be brought about by these minute animals. From his many observations, he concluded that many oribatoid mites were spore carriers. Their bodies, especially small projections, bristles, and the mouth parts, are often infested with fungus spores. Many species have the habit of climbing higher plants, from which, one may assume, they are washed down with strong rain. Later when it is dry, they again climb up and infect another plant. They also can produce minute injuries to the plants with their mouth parts and so it is possible that the disease germs of plants are directly inoculated. Stunkard (1937, 1940, 1941) established that the oribatoids may act as the intermediate hosts of certain anoplocephaline cestodes.

[1]This paper is based on a thesis done under the direction of Professor H. W. Stunkard and submitted to the Department of Biology and the Faculty of the Graduate School of Arts and Sciences of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2]The writer gratefully acknowledges the assistance of Dr. H. W. Stunkard whose encouragement and inspiration made the successful completion of this project possible, and of Dr. D. Ludwig, then of the Biology Department of New York University, and of Dr. E. W. Baker, of the Entomology Research Branch, U. S. Department of Agriculture.

Like most Acari, the oribatoid mites leave the egg as six-legged larvae and in the course of subsequent development pass through four molts. After the first molt, they are in the eight-legged nymphal stage (protonymph) and then pass through two further nymphal stages, the deutonymph and tritonymph. The adult form is reached in the fourth molt. Each molt is initiated by a quiescent stage which can be of different lengths.

Ontogeny occurs by a very gradual development from the egg to the sexually mature adult stage. All stages nourish themselves in a similar fashion and also carry on the same modes of life. It consequently requires little adaptation from the larval state to meet conditions of mature life and therefore the immature stages, with few exceptions, greatly resemble the adults. It follows that one of the best aids in the identification of the immature stages of oribatoid mites is comparison with the adult. An acarologist examining for the first time the protonymphs of *Hypochthonius rufulus* Koch, Family Hypochthoniidae, or *Nothrus palustris* Koch, Family Camisiidae, would have little hesitancy in correctly cataloging them as to family.

Grandjean (1933a, 1933b, 1934, 1949) stated that formulae representing the setae carried on certain parts of the body of the Oribatei give in the most concise form the exact information necessary for the determination of genera and species. It is axiomatic that the number and placement of bristles are of great importance in identifying Acari. However, the use of any one isolated character in the specific determination of the oribatoid mites is unwise and would lead to confusion. For example, without questioning materials, methods, or the validity of results but applying the formulae (Grandjean, 1949), it is seen that four other genera in addition to *Galumna* Heyden have the same number of bristles. Obviously it would be difficult to determine accurately by this method alone the species of a developmental stage of a galumnid mite. The use of accurate figures, coupled with detailed description, is still the only reliable method (excluding comparison with type collections) for the identification of unknown species.

Michael (1884) published incomplete data on the length of the developmental stages of four different species of oribatoid mites. Temperature and humidity were not controlled and his results were inconclusive, although they did firmly establish the immature stages as larva, protonymph, deutonymph, and tritonymph. For *Liacarus palmicinctum* (Nicolet), Family Liacaridae, the only species of which he timed the various stadia (and he cited only one individual), the total time was one year and ten days. This is at great variance with observations made during this investigation, and those of Stunkard (1944). No drawings of the immature stages were included.

Stunkard (1944) raised *Galumna* sp., Family Galumnidae, from egg to adult under laboratory conditions at a constant temperature of 25° C. and a relative humidity of approximately 82 percent. From the variability of his results, it is probable that he was dealing with more than one species. As this report was merely an abstract of results there were no accompanying figures or descriptions.

Until the present no one has either reared a single species of an oribatoid mite in numbers great enough to give valid results, or figured

the immature stages so that isolated specimens collected from the field could be identified accurately. Three species of *Galumna* have been cultured successfully in the laboratory. These species, *G. nervosus* (Berlese), *G. longipluma* (Berlese) and *G. elimatus ithacensis* Jacot, were used not only because of their roles as intermediate hosts of the anoplocephaline cestodes but also to elaborate on the previous work of Stunkard. From environmental studies on this genus (Jacot, 1937a; Sengbusch, 1951), it was obvious that a high humidity had to be maintained at all stages of the life cycle. To obtain uniformity in the results, a constant temperature of 25°C. was used throughout the experiment except where indicated elsewhere.

The taxonomy of the oribatoid mites is still controversial, but the following outline is the one listed by Vitzthum (1931) in his monumental work on the Acari, by Radford (1950) in his systematic checklist of mite genera and type species, by Baker and Wharton (1952) in their recent review of acarology, and is the one followed today by most acarologists:

Acarina *Nitzsch*

Order *Acarina Nitzsch*
Suborder *Sarcoptiformes Reuter*
Supercohors *Oribatei Duges*
Family *Galumnidae Grandjean*

This study involves a consideration of the following: first, the development of a technique whereby free-living mites can be reared in the laboratory; second, the size and shape and the length of time involved in the various stages of development of three species of oribatoid mites; and third, observations on the biology of oribatoid mites.

MATERIALS AND METHODS

1. *Culture Chambers*

After much experimentation, a technique was evolved which not only provided for the culturing of large numbers of adults, but also for the rearing of isolated eggs, larvae and nymphs. This procedure has been used successfully by the writer in raising acarid mites, as well as oribatoid mites, and it is suggested that it could be employed advantageously in the culture of any free-living mite.

Kates and Runkel (1948) kept oribatoid mites in the laboratory in 50 cc. weighing bottles containing small pieces of filter paper moistened with a few drops of water. Mites kept in these tightly stoppered bottles lived for several weeks but the rate of mortality was very high. This type of container was tested but abandoned, not only because of the mortality involved, but because the height and narrowness of the bottles interfered with close, accurate microscopic observation.

Stunkard suggested (personal communication) the use of small Carrel tissue-culture flasks. Filter paper was placed on the bottom of these bottles, moistened, and the small neck stoppered with a cotton plug. These flasks were then placed in Scheibler desiccator jars containing a saturated solution of $ZnSO_4$ to provide a relative humidity

of 82 percent (Ludwig and Landsman, 1937). Mites and food were inserted into the narrow neck of the flask by means of a small camel's hair brush. This method was discarded because the mold growth was too difficult to control, individual mites and eggs could not be removed without great difficulty, and moisture collecting on the inside of the jars made microscopic observation impossible.

Jacot (1937b) recommended a cell formed by a glass ring mounted on a microslide for the culture of non-predacious, non-parasitic mites. This method provided the best clue to the difficult problem of rearing the immature stages, but it is impracticable for maintaining more than three or four adults in one container.

After the mites were collected, containers were needed in which to keep sufficient numbers of each species to insure the deposition of large numbers of eggs. Stender jars having an outside diameter of 50 mm. and a height of 30 mm. provided the best answer to the problem of maintaining adults. Filter paper was placed on the floor of the jar and an attempt was made to keep it constantly moist. Although mites of most species can climb the smooth glass walls of the jar, the heavy ground-glass cover was sufficient to prevent their escape. These stenders were placed in Scheibler desiccator jars having an inside diameter of 150 mm. and containing a saturated solution of $ZnSO_4$ in their bases. This was necessary to prevent too rapid evaporation of the water from the filter paper. As long as the filter paper remained wet the relative humidity within the container approached saturation, but if there was loss, the relative humidity could not fall below that of the desiccator. To insure a constant temperature of 25° C., the desiccators containing the stender jars were placed in an incubator.

Adults, having been collected by means of a modified Tullgren apparatus (Sengbusch, 1951), were isolated by species in separate stender jars. These dishes were then examined daily under a binocular dissecting microscope using a magnification of 30 times. As soon as the eggs were discovered they were placed, by means of a small camel's hair brush, in microcells. These microcells were modified from the description given by Jacot (1937b). Ordinary 3″ x 1″ microslides were used and to them mounting cells were fastened with a viscous mixture of poly-vinyl alcohol (PVA). Two different sized cells were tried. One having an outside diameter of 1.5 mm. and 0.3 mm. in height, was found to be excellent in which to keep eggs and larvae; a larger size with an outside diameter of 1.8 mm. and 0.5 mm. in height provided more room for the larger, more robust nymphs and adults. Etching was never found to be necessary to provide traction for these animals as most species can traverse the sheer glass side of the container with ease. Blotting paper was tried on the floor of the cells but it offered too great a barrier to the weak newly hatched individuals, so filter paper was used. The filter paper was cut so that it fitted snugly at all edges to prevent the mites from getting underneath where they could not be observed. The paper was moistened almost daily to provide the water which is so necessary for this group of mites. It was found that placing the opened cell on the stage of the microscope for observation under a lamp caused most of the included moisture to disappear, thus necessitating frequent dampening.

Ordinary No. 1 coverslips, 7/8" square were used as covers. A very thin layer of vaseline was carefully spread on the outer edge of the cell and the coverslip pushed in place. No attempt was made to make this an air-tight seal as these rearing cells were then placed in desiccators containing the $ZnSO_4$ solution to provide the same humidity as for the stenders. The vaseline was used instead to keep the space between the cell and the cover small enough to prevent the escape of the inhabitants. Great care had to be taken not to use an excess of the vaseline as the immature mites would soon locate it and become hopelessly mired.

2. *Nutriment*

There was no feeding problem with the adult oribatoid mites as Jacot (1937b) offered a number of suggestions, any one of which would do for the galumnid mites. However, the culture of the immature stages was another matter. The material which furnished good sustenance for the adults always had one or more drawbacks for the larvae and nymphs. Moss on which the adults would stay and feed would be deserted by the larvae, who would wander off and die. The newly hatched individuals were also apathetic to such materials as dead wood and lichens. Mold of any sort soon entangled the youthful members and had to be eradicated stringently. Care had to be taken not to provide any hiding places for the subjects as daily observation was obligatory. Therefore, a long succession of possible foodstuffs, including appleskin, potato, germinating wheat seeds, rice, oatmeal, yeast, sugar water, and malt, was tried with little or no success.

Since it is evident that these minute species need a food which is abundant and readily available in their normal habitat, a piece of bark covered with *Protococcus*, a green alga, was brought into the laboratory. With a dissecting needle small pieces of this alga were loosened from the bark, picked up with a moistened camel's hair brush and placed in rearing cells containing newly emerged larvae. The following day the larvae were observed busily feeding on this material, and through their semi-transparent exoskeletons, patches of dark green were seen in the alimentary tract. *Protococcus* was then used throughout the experiment as food for the immature galumnids and from the results in Table VI there is good evidence that this material provides the necessary requirements for survival.

In the course of this study, oribatoid mites other than galumnids were cultured. While the technique outlined is in general admirably suited for this purpose, the results indicate that although these animals inhabit similar environmental niches, they are relatively specific in the food to which they are attracted. For example, the immature stages of *Melanozetes miridianus* Sellnick, Family Ceratozetidae, which is closely related to the large-winged mites, were never observed feeding on *Protococcus* but exhibited a slight preference for decaying vegetable matter. Another species, *Tryhypochthonius badius* (Berlese), Family Hypochthoniidae, one of the primitive oribatoid mites, also was seen continuously on decayed moldy moss, especially if this material was thoroughly saturated. Their short powerful legs enabled this species to move through this type of terrain which would have completely

entangled the galumnid mites. *Nothrus rugulosus* Banks and *Nothrus palustris* Koch, Family Camisiidae, were also maintained for short periods of time. These arboreal species, which normally live underneath the bark of trees, were never actually seen chewing on bits of soft dead wood, but were always found in close proximity to them. Therefore, on the basis of incomplete results it is seen that of the four different genera of oribatoid mites in which attempts were made to culture the immature stages only one, *Galumna*, responded successfully to *Protococcus*.

OBSERVATIONS

1. *General*

Oviposition in species of *Galumna* may occur in nature from spring to fall and in the laboratory is probably correlated with temperature. In 1948, eggs were laid in April and May and as late as the end of October. Initial oviposition for the three species of oribatoid mites studied in the present investigation occurred shortly after being brought into the laboratory. Moss was collected on Nov. 15, 1950 from a wooded area along a small stream near Hyde Park, New York and placed in a modified Tullgren apparatus (Sengbusch, 1951). The following day the two species which appeared most numerous, *Galumna elimatus ithacensis* and *Galumma nervosus*, were separated and placed in stender dishes which were kept in a constant temperature incubator at 25° C. These dishes were examined daily for the presence of eggs. On November 24, 1951, seven *Galumna elimatus ithacensis* eggs were discovered and immediately placed in small culture cells for observation. This species continued to lay eggs until Dec. 4. On Dec. 8, *Galumna nervosus* eggs were found and these were laid at intervals until Dec. 23rd. Also during December of that same year another staff member discovered a group of mites in a snail terrarium. They proved to be *Galumna longipluma* and were placed in stender dishes for observation. On Dec. 15, five eggs were found in a cluster. This was not repeated until Feb. 4 when sixteen eggs were discovered in one clump under the filter paper and adhering to the glass dish. It seems unbelievable that one female could carry that many eggs within her body and yet the alternate hypothesis, that three or four females selected the exact same spot for oviposition is also unlikely.

There is considerable variation in the number and the location of eggs deposited by oribatoid mites. The number of eggs deposited by *Galumna longipluma* generally varied between four and five, which agrees with the observations of Jacot (1934), who stated that the largest number of eggs observed in a female was five. *Galumna elimatus ithacensis* produces more ova, so that between six and eight eggs were deposited. *Galumna nervosus* usually laid seven or eight eggs in a clump. These eggs were generally found in a heterogeneous group dependent upon the type of material the female had selected in which, or on which, to deposit her eggs. In many cases the white shiny eggs were located on the glass side of the stender dish below the edge of the filter paper. The eggs when laid are covered with a thin coat of a viscous material which tends to keep them clumped and adherent

to each other and to the substrate. The female galumnid mite usually seeks a secluded area in which to insert the long ovipostor and extrude the eggs. If that area is large enough, the whole complement of eggs will be found there. If, however, the space is too small the female will seek another nearby locale and continue the oviposition. For example, in stenders containing the common moss, ***Bryum bimum***, a single egg would be found hidden in the axil of a leaf with the nearby axils having the remaining eggs. In many cases the female would use old exuviae in which to hide her offspring. The *Nothrus* female seemed less concerned with secrecy and her one to two eggs were generally found sticking to the underside of a piece of rotten wood. ***Pseudotritia ardua*** (Koch), Family Phthiracaridae, used the tri-valved tip of the ovipositor to spread apart the fibers of the filter paper and to insert the egg therein. These eggs were never found in clusters but always within a small area.

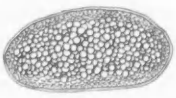
Some oribatoid eggs do not resemble those of the galumnid mites. Jacot (1930b) figured the egg of ***Pseudotritia ardua***, which has a relatively thick shell with prominent ridges possessing hook-like projections at one end and a deep brown coloration. All the eggs of the phthiracarinid group, which have been studied during the preparation of this report, possess these same characteristics. The eggs of *Nothrus rugulosus* and *N. palustris* have the same general shape as those of the galumnids, but have a more greyish appearance coupled with slight elevations to which bits of debris adhere. It is interesting to note that the eggs of *Tyrophagus lintneri* (Osborne), Family Acaridae, resembled more closely those of the galumnids than any of the other species of oribatoid studied.

There is some possibility that these mites are wholly or in part parthenogenetic. Grandjean (1947) attempted to demonstrate parthenogenesis for *Nothrus palustris*, *N. silvestris* Nicolet, and *Nanhermannia nana* (Nicolet), Family Nanhermanniidae. However, he originally isolated tritonymphs of these species and there is the possibility that these late nymphal females had been inseminated by mature males before being isolated, as occurs frequently in the parasitic and epizoic Acaridiae. The writer has raised single eggs to adulthood in the three species of *Galumna* studied with no evidence of parthenogenesis.

2. *Description of Developmental Stages Galumna elimatus ithacensis* Jacot

Figs. 1–15

Egg (Figs. 1 & 2).—The eggs of galumnid mites are typically oval to ovate, and have one side flattened as do those of many insects. Eggs recently laid have a whitish almost translucent appearance, which makes them relatively easy to locate in the small culture cells. Within three or four days development becomes evident externally with an amber pigmentation appearing at both poles. From material on slides it is evident that the egg when laid contains a mass of large yolk globules. Within five days the typical segmented arthropod embryo with stomodaeum and proctodaeum is visible even though the yolk material tends to obscure the details. The thin shell is al-



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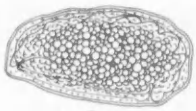

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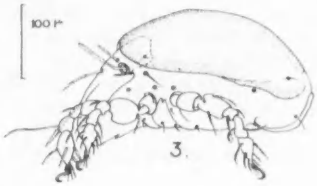

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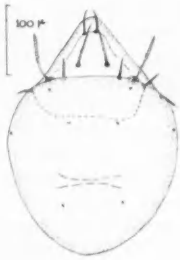

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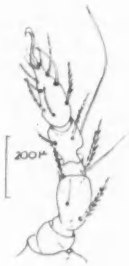

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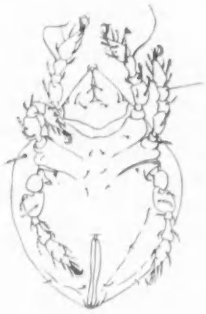

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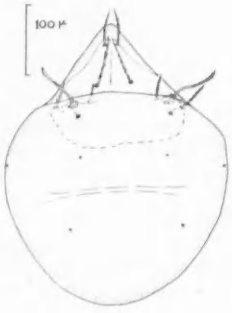

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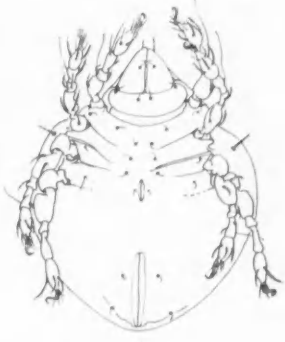


9.

Details of the development of *Galumna elimatus ithacensis*.

FIG. 1. Egg, undeveloped. FIG. 2. Egg, five days old. FIG. 3. LARVA, side view. FIG. 4. Larva, dorsal view. FIG. 5. Larva. Leg I, ventral view. FIG. 6. Larva, ventral view. FIG. 7. Protonymph, dorsal view. FIG. 8. Protonymph. Leg I, ventral view. FIG. 9. Protonymph, ventral view.

most transparent and slightly elastic so that major variations in form and color are noticeable with only low magnification. A day or two before hatching, the outline of the larva can easily be perceived using a magnification of 60 times. Just before emergence the exoskeleton of the larva becomes totally pigmented with the amber color noted above. At this time the legs are curved ventrally with the unguiculi almost touching the midventral line. The pseudostigmatic organs and the bristles of the cephaloprothorax are bent down anteriorly, lying on top of the dorsal surface of the exoskelton.

Measurements of several eggs are given in Table V. Differences in size and proportion can be noted, but it is doubted if they are of any diagnostic value with the exception of placing the individual within one of the three general size groups of adult North American galumnids (0.3, 0.5, 0.7 mm.).

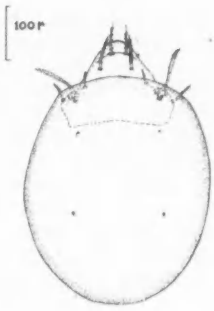
Larva (Figs. 3–6).—In order to describe the various parts of these highly complex, though minute organisms, it is necessary to give names to these parts. Jacot (1937a) presented a complete index of terms for this group and as he is the recognized authority for the Galumnidae that terminology will be followed in the remaining discussion.

The shape, as in the adult, is broadly ovate; cephaloprothorax or aspis, is relatively long, broad and conical; rostrum is narrow, but not prominent; pseudostigmatic organs are cylindrical to slightly flattened, fairly long, slender, without a distinct head, pointed with barbs; pseudostigmata arise beneath the anterior overlap of the notogaster and are bounded on either side by a pair of prominent barbed bristles; lamellar and rostral bristles inserted dorsally on the aspis; exoskelton has a slight yellow-brown pigmentation.

The notogaster is broad, wider anteriorly and narrowing posteriorly until it overhangs and is rounded onto the ventral plate (Fig. 3). There are three pair of prominent mesonotal insertions; the first corresponding to the anterior porose area of the adult; the second in the approximate area of the mesal adalar insertions; and the last, posterior and laterad to the anal plate. Pteromorphae are not present and do not appear until after the fourth molt.

The ventral plate is elliptical, pointed posteriorly; gular bristles prominent and very laterad; sternal bristles I long and protruding anteriorly over the labium; three pairs of apodeme bristles prominent; no genital plate; anal plate large and extending caudad and dorsad to meet the notogaster. Genital and anal covers do not appear until the adult emerges.

Fig. 5 illustrates leg I in ventral aspect showing the extent and position of bristles. Jacot (1937a) stated that there are enough specific characters on the body to obviate the use of leg characters and doubted whether the legs of these species, if found separated from the body, could be specifically determined. Nevertheless, it is pertinent to note that the unidactylate unguiculus is consistent throughout the immature stages of this group of mites, not becoming triheterohamate until adulthood is reached. By comparing Fig. 5 with leg I of the protonymph, deutonymph, and tritonymph (Figs. 8, 11 & 14), it is evident that there is no regression of setae during ontogeny, only additions which augment the original complement as the animal in-



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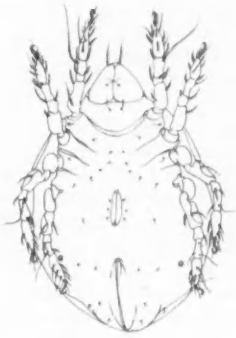

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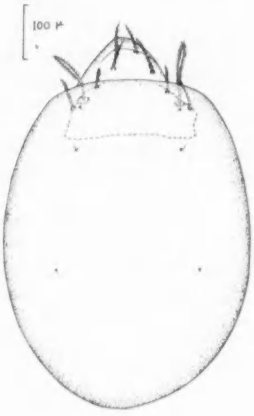

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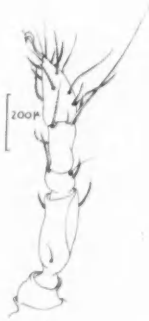

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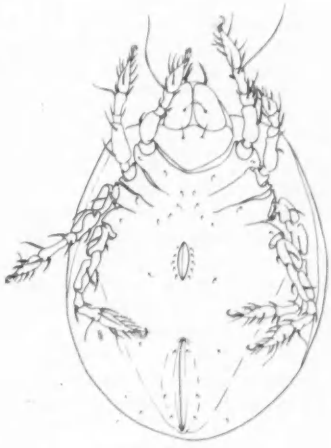


15.

Details of the development of *Galumna eliminatus ithacensis*.

FIG. 10. Deutonymph, dorsal view. FIG. 11. Deutonymph. Leg I, ventral view. FIG. 12. Deutonymph, ventral view. FIG. 13. Tritonymph, dorsal view. FIG. 14. Tritonymph. Leg I, ventral view. FIG. 15. Tritonymph, ventral view.

creases in size and complexity. Although no interspecific differences were noted, leg characters may be used to determine generic rank.

Protonymph (Figs. 7–9).—As has been stated above the development of oribatoid mites is gradual with all stages bearing a marked resemblance to one another. Since this is so, a complete description of each stage would be repetitious to a great extent. Therefore, in the remaining accounts the discussion will be limited to additions, regressions, and or movements, which are deemed important. There is a definite increase in size from stage to stage, as recorded in Table V, and this relationship will be discussed more fully later.

Dorsally there is little difference between the larva and the protonymph, so it is on the venter that major differentiation is to be noted. First, a fourth pair of legs has been added. These legs may be readily distinguished on the protonymphs of all the oribatoids because of the paucity of bristles, only the tarsi having two rows of setae. With the presence of a fourth pair of legs there is also the concurrent addition of another pair of apodeme bristles. Second, a genital area now appears and is bounded midlaterally by a single pair of bristles. These two genital setae are diagnostic for a great many oribatoid protonymphs (Figs. 9, 16, 17, 23) as Grandjean (1949) has indicated in his genital formula. Third, the posterior lateral bristles of the larva have migrated to the anal area or are replaced by a pair of anal bristles. Such a change in position is probably due to the appearance at this stage of a new segment, the adanal (Grandjean, 1949).

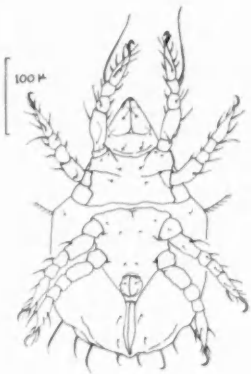
Deutonymph (Figs. 10–12).—At this stage the length of the notogaster becomes proportionately greater in relation to that of the cephaloprothorax, indicating that most of the growth has occurred in the abdominal area. One of the characteristics of the deutonypmhs of this group of mites is the increase in size of the genital area, with the concomitant increase in the number of bristle insertions to six (Fig. 12). The paramesal bristles also appear now. According to Grandjean (1949) a second new segment, the anal, appears at this second nymphal stage and borders the anal opening. There is evidence for this proposal in the presence of a third pair of paranal bristles.

Tritonymph (Figs. 13–15).—Again there is a proportional increase in the size of the abdomen in relation to that of the cephaloprothorax. With the exception of the general increase in size to which reference has already been made, it is on the venter that distinguishing characters for this stage appear. The genital plate now bears five pairs of setae and this formula holds true for all the galumnid tritonymphs studied. With this exception there seems to be no further additions of bristles on the body of the mite at this time.

EXPLANATION OF PLATE III

Details of the development of *Hypochthonius rufulus, Nothrus palustris, and Galumna nervosus.*

FIG. 16. *H. rufulus.* Protonymph, ventral view. FIG. 17. *N. palustris.* Protonymph, ventral view. FIG. 18. *G. nervosus.* Larva, dorsal view. FIG. 19. *G. nervosus.* Larva, ventral view. FIG. 20. *G. nervosus.* Molting larva, dorsal view. FIG. 21. *G. nervosus.* Molting larva, ventral view.



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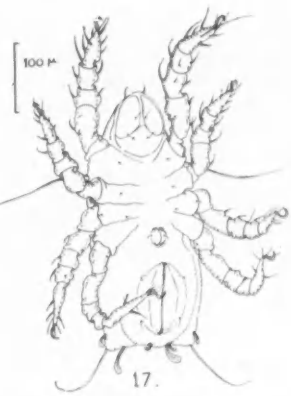

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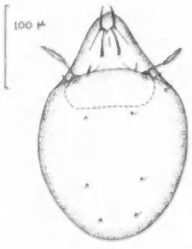

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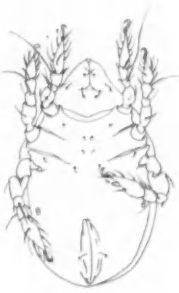

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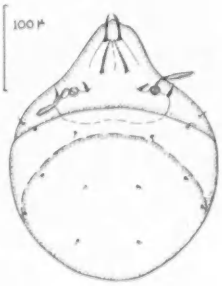

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21.

From Table I, it is evident that there is much variation in the duration of development in this species. From the egg to the adult, seventy-two to one hundred and seven days were required in the fourteen specimens which reached maturity. This same type of deviation occurs in all the stages and will be alluded to later. It is noticeable however, that there is a marked consistency in the average times for the emergence of the larva, protonymph, deutonymph and tritonymph. It seems probable that with a large enough sampling and with the environmental conditions approaching optima, these times would be constant. It is likely that the greater length of time necessary in the tritonymph stage is due to the greater changes, such as the addition of the pteromorphae, before the last molt is completed.

TABLE I

LENGTH OF TIME IN DAYS AT 25° C. OF THE DEVELOPMENTAL STAGES OF *Galumna elimatus ithacensis*

EMERGENCE OF LARVA	EMERGENCE OF PROTONYMPH	EMERGENCE OF DEUTONYMPH	EMERGENCE OF TRITONYMPH	EMERGENCE OF ADULT	TOTAL
11	11	14	20	36	92
14	23	25	9	14	85
12	13	13	19	46	103
12	12	12	18	36	90
12	12	12	20	35	91
19	9	12	21	18	79
19	11	16	17	20	83
20	24	15	16	15	90
15	18	12	11	16	72
22	10	11	11	19	73
13	15	10	9	27	74
13	16	10	9	35	83
13	24	27	21	15	100
13	28	30	21	15	107
Ave. 14.9	Ave. 16.1	Ave. 15.6	Ave. 15.9	Ave. 24.8	Ave. 87.3

Galumna nervosus (Berlese)

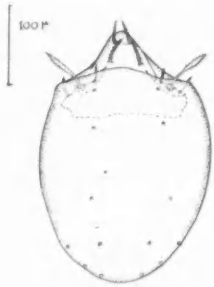
Figs. 18–27

From a perusal of the figures of the developmental stages of the two species of galumnid mites included in this study, it can be readily ascertained that the similarities greatly outnumber the differences. Grandjean's work indicates that it is impossible to distinguish one species from another of the same genus using the characters of the

EXPLANATION OF PLATE IV

Details of the development of *Galumna nervosus*.

FIG. 22. Protonymph, dorsal view. FIG. 23. Protonymph, ventral view. FIG. 24. Deutonymph, dorsal view. FIG. 25. Deutonymph, ventral view. FIG. 26. Tritonymph, dorsal view. FIG. 27. Tritonymph, ventral view.



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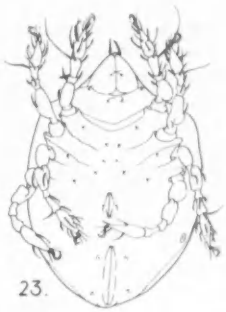

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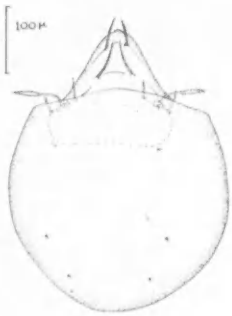

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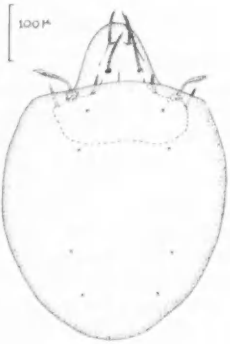

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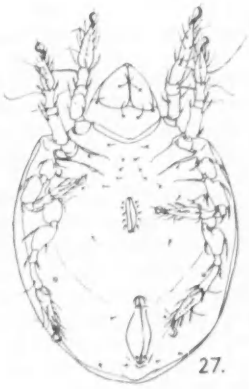

27.

immature stages. On the basis of the work completed in this investigation (it is realized that this covers only two species out of the twenty-five or so which have been recorded from North America) it seems that there are minute specific distinctions, which if described accurately would enable systematists, not only to correctly identify individuals, but would greatly aid in the determination of relationships and evolutionary import. Since the similarities between the the ontogenies of *G. elimatus ithacensis* and *G. nervosus* are so manifold the following descriptions will be limited, for the sake of briefness, to distinguishing characters.

TABLE II

LENGTH OF TIME IN DAYS AT 25° C. OF THE DEVELOPMENTAL STAGES OF *Galumna nervosus*

EMERGENCE OF LARVA	EMERGENCE OF PROTONYMPH	EMERGENCE OF DEUTONYMPH	EMERGENCE OF TRITONYMPH	EMERGENCE OF ADULT	TOTAL
10	10	7	8	12	47
11	8	7	8	11	45
10	8	8	10	12	48
10	7	8	9	11	45
10	8	9	8	13	48
12	8	9	9	11	49
10	8	8	8	11	45
10	8	8	9	12	47
10	9	8	8	10	45
11	8	8	8	11	46
11	9	8	9	11	48
11	9	8	9	12	49
12	8	8	9	13	50
12	8	8	8	13	49
11	8	7	8	12	46
Ave. 10.7	Ave. 8.3	Ave. 7.9	Ave. 8.5	Ave. 11.7	Ave. 47.1

Egg.—From Table V it can be seen that while the length of the egg of *G. nervosus* is less than that of *G. elimatus ithacensis*, the width remains the same. Therefore the proportions are different, so that this egg appears shorter and more round than that of *G. elimatus ithacensis*.

Larva (Fig. 18–21).—The shape is ovate as in the adult; cephalothorax very broad; rostrum prominent, ending anteriorly in a well defined nubble; pseudostigmatic organs long, slightly clavate with barbs, and bending sharply laterad near the head; notogaster broadly triangular having the anterior margin relatively straight.

The only accurate method of determining the number and position of the dorsal bristle insertions is to make slide preparations of molting individuals. As has been stated a quiescent period initiates all molts. Just before the mite enters on this static state, the conjunctiva opens out (Fig. 20). This non-pigmented conjunctiva seems to be folded down like a figure Z during most of the time of active feeding. The

presence of this light band between the aspis and the notogaster always indicates the initiation of molting. The quiescent period generally lasts from two to three days in the case of the first three molts and four to five days for the last molt. At the expiration of this time, the conjunctiva between the notogaster and the ventral plate splits. This process begins near the anal plate and proceeds anteriorly as the mite emerges backwards. At the completion of exuviation, the only point of attachment of the notogaster to the rest of the cast skin is at its juncture with the aspis. This conjunctiva not being as heavily sclerotized as the rest of the exoskelton acts as a hinge. Thus the notogaster of the exuvia can be easily lifted up to afford the female an ideal place in which to hide her eggs. Slides of cast skins also afford the investigator a method of studying the external characters of the immature stages without the necessity of interrupting timed developmental series to kill and mount specimens at each stage of development. Figs. 20 & 21 show the expanded larva as it appears before the protonymph emerges. It is at this time that most of the growth of the animal occurs. When the mite is in this dilated condition, bristles that normally would be obscured by the overhang of the notogaster, are shown in their true relationship. Thus it can be seen that there are nine pairs of insertions on the notogaster, of which the three most caudal pairs can be identified on the ventral view. On most of the figures the lateral insertions are omitted because these bristles cannot be seen from a true dorsal or ventral view. These insertions are very minute which made a magnification of 1140 times necessary for this type of work.

Protonymph (Figs. 22, 23).—Dorsally this stage can be distinguished from that of *G. elimatus ithacensis* by the more flattened leading edge of the notogaster, by the more clavate laterally bending pseudostogmatic organs, and by the presence of an additional pair of mesonotal bristles. Ventrally this species lacks a bristle on parasternum I.

Deutonymph (Figs. 24, 25).—The relationships already outlined continue to hold true with the addition that the bristle of parasternum IV and bristle of apodeme IV are much closer than they are in *G. elimatus ithacensis*.

Tritonymph (Figs. 26, 27).—The only difference, excluding the ones already mentioned which continue to maintain themselves once they became established, is the more posterior position of the paranal insertions.

DISCUSSION

From the observations of Stunkard (1944) and from field studies conducted during this research, it is known that oribatoid mites in their natural habitat deposit eggs at least twice a year namely, once in spring during the first weeks in May, and again in the fall around the end of September and the early part of October. Temperature seems to be one of the controlling factors in this process. The following explanation, therefore, could elucidate the discrepancy between the time of occurrence of oviposition in the laboratory and in the field.

First an attempt was made to learn the effect of freezing temperature on galumnid eggs. Nine eggs were placed in culture cells

which were sealed with vaseline to keep the humidity constant, and placed in closed petri dishes in a shady spot outdoors. This was done for one month beginning Jan. 20 when the temperature went below freezing almost every night. At the expiration of this period the eggs were brought into the laboratory and placed in an incubator at 25° C. One month later, March 20, all of these eggs were shrivelled and dead. While the results of this experiment are not conclusive, they tend to show that galumnid eggs can not withstand the rigors of winter.

Second, frozen material brought into the laboratory for observation always contained viable nymphs. Thus it is shown that these immature stages are relatively cold hardy.

Therefore it is suggested that the galumnids lay their eggs outdoors early in the fall. These eggs hatch before frost develops so that they survive the winter as nymphs. The adults which have not matured enough for oviposition by the time the ground is frozen retain their eggs until the temperature rises again in the spring. It was probably some of these latter females which when brought into the warm laboratory responded to the spring-like conditions and deposited their eggs.

TABLE III

LENGTH OF TIME IN DAYS AT 20° C. OF THE DEVELOPMENTAL STAGES OF *Galumna nervosus*

EMERGENCE OF LARVA	EMERGENCE OF PROTONYMPH	EMERGENCE OF DEUTONYMPH	EMERGENCE OF TRITONYMPH	EMERGENCE OF ADULT	TOTAL
15	14	14	13	14	70
14	18	10	8	13	63
14	12	14	7	12	59
15	17	9	8	11	60
Ave. 14.5	Ave. 15.3	Ave. 11.8	Ave. 9.0	Ave. 12.5	Ave. 63.0

From Table III it can be seen that lowering the temperature from the constant 25° C., at which most of the experiments were run, to room temperature of approximately 20° C., caused a definite lengthening of the duration of the developmental stages of *Galumna nervosus*. Thus it is indicated that the members of this genus would probably take a greater amount of time to develop to sexual maturity in their natural environment than that which occurred in the incubator. From the preceding data and the results of Tables I, II and IV, it is possible to approximate the life history of the galumnids in the New York area.

Assuming eggs are laid May 1, the development would approximate that of the laboratory, but would be slightly longer because of the low temperatures encountered in this area in the spring (ave. temp. for May being about 16° C.). Thus for *Galumna elimatus ithacensis* the emergence of the adults could be postulated in about four months or about Sept. 1. These adults would begin to lay eggs in another month or about Oct. 1, which agrees with the evidence from field ob-

servations. Most of these eggs would reach the nymphal stage before the first frost. Although there is no evidence for this it seems quite logical that the low temperatures of winter would cause either a cessation of growth or minimum of development. Therefore these nymphs, having the length of time before they reached sexual maturity extended by the three cold months, would not emerge as adults until April 1. Allowing another month before oviposition, eggs would again be laid on or about May 1.

More evidence needs to be gathered before the exact effects of raising and lowering the temperature on the length of the stadia of galumnids is known. It is logical to assume that different species have different optima. It is known for insects (Ludwig, 1928) that the nearer to the optimum temperature that development occurs the more stable and consistent are the lengths in time of each stage. From Table I it can be seen that there is wide variation in the develop-

TABLE IV

LENGTH OF TIME IN DAYS AT 25° C. OF THE DEVELOPMENTAL STAGES OF *Galumna longipluma*

EMERGENCE OF LARVA	EMERGENCE OF PROTONYMPH	EMERGENCE OF DEUTONYMPH	EMERGENCE OF TRITONYMPH	EMERGENCE OF ADULT	TOTAL
11	10	11	12	14	58
11	11	10	12	14	58
11	11	11	11	16	60
11	11	11	12	15	60
12	10	11	12	15	60
10	11	12	14	14	61
10	11	13	14	16	64
10	11	13	14	18	66
Ave. 10.8	Ave. 10.8	Ave. 11.5	Ave. 12.6	Ave. 15.3	Ave. 60.9

ment of *G. elimatus ithacensis* at 25° C., and from Tables II, III and IV that there is a much closer correlation in the development of *G. nervosus* and *G. longipluma*. Jacot (1937a) stated, "*G. nervosus* and *G. longipluma* are certainly recent introductions in America because they are found chiefly about our cities, and have no subspecies in America", and that *G. elimatus ithacensis* is an American subspecies of an European species. If this is true then the wide ranges of time in the development of *G. elimatus ithacensis* are due to the fact that 25° C. is above the average mean temperature of this region, and therefore may be above the optimum range for the development of this indigenous subspecies. At the same time it seems probable that the recently introduced species came from a warmer clime, and have not as yet completely acclimated themselves. In other words, 25° C. is probably nearer the optimum temperature for *G. nervosus* and *G. longipluma* than it is for *G. elimatus ithacensis*. Further experimentation in rearing oribatoid mites at a series of different temperatures seems indicated before any further statements are made as to their optimal conditions for development.

TABLE V

MAXIMUM AND MINIMUM

MEASUREMENTS OF DEVELOPMENT STAGES (in Micra)

	EGG		LARVA		PROTONYMPH		DEUTONYMPH		TRITONYMPH	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
Galumna elimatus ithacensis	265–250	133–117	351–304	250–211	421–351	335–250	523–460	398–351	694–585	507–468
Galumna nervosus	195–183	133–130	304–234	242–148	343–312	304–228	421–390	351–273	577–540	437–390

Table VI presents a breakdown of the number of individuals which were able to survive each stage during the course of this experiment. From a total of ninety-eight eggs which were collected, ninety-two hatched into viable larvae, a fact indicative of low infertility. It is in the larval state, however, in which the second highest mortality rate (approximately 22%) is encountered. It is logical to assume that at this stage, when the individual is smallest and weakest, the mite would have great difficulty in adjusting to the environment. Also, the greatest loss occurred in *G. elimatus ithacensis*, the first species to hatch during this study. Therefore it is possible that the technique in handling the culture cells was not at this time as well perfected as it was for the other two species, and it is quite likely that in future experiments the number of individuals surviving this stage would be proportionately greater. The amount of loss for the next two stages, protonymph and deutonymph, was relatively constant at about 15%.

TABLE VI

NUMBER OF SPECIMENS WHICH SURVIVED EACH MOLT

	EGGS	LARVAE	PROTONYMPH	DEUTONYMPH	TRITONYMPH	ADULTS
Galumna elimatus ithacensis	42	40	28	23	19	14
Galumna nervosus	37	36	30	25	23	19
Galumna longipluma	19	18	14	12	11	8
Total	98	92	72	60	53	41

During the time of the fourth and last molt the highest percentage of deaths occurred. This was not entirely unexpected as it is at this time that the individual undergoes its major differentiation into the adult. This molt is also marked by a period of non-feeding which almost doubles that of the other stages. The overall percentage of survival of 42% indicates that the materials and methods developed for the culture of galumnid mites is successful.

Table V. lists the maximum and minimum measurements of the immature stages of *G. elimatus ithacensis* and *G. nervosus*. Unfortunately not enough specimens were available for measurements so that a complete statistical analysis could be made. However from these data, it can be seen that the growth in length between successive stages seems to follow a geometric progression. Beginning with the average length of the larval stages of both species and increasing the length by one quarter for each successive stage, one will obtain results which fall within the actual ranges as recorded in the table in all but one case. This indicates that Brook's Law (essentially, that the length of any stage when multiplied by the factor 1.25 will give the length

of the ensuing stage) applies to the development of the immature stages of galumnid mites.

Also from Table V it can be noted that the immature stages bear the same relationships to one another as do the adults. For example, the adult of *G. nervosus* averages 0.58 mm. in length, which is approximately 4/5 that of *G. elimatus ithacensis* and that this ratio is maintained, within wide limits, throughout the ontogeny of these mites.

It is regretted that the number of species to be compared was so few. However this is the first successful attempt to culture an oribatoid mite. As the technique improves in the rearing of these animals more individuals will be available for study. Then normal variation within a species can be determined and more species within a genus can be investigated so that a practicable key for the identification of the immature stages can be formulated.

SUMMARY

1. A method was developed that can probably, with modifications, be used successfully to culture any free-living mite.

2. For the first time, three species of oribatoid mites were timed in their development from egg to adult.

3. Descriptions and figures of the immature stages of *Galumna nervosus* (Berlese) and *Galumna elimatus ithacensis* Jacot were made.

4. It is shown that lowering the temperature for the development of *Galumna nervosus* (Berlese) from 25° C. to 20° C. increases the time necessary for the emergence of the adult by approximately two weeks.

5. An hypothesis on the life history of the galumnid mites in nature is stated.

6. It is noted that Brook's Law probably applies to the growth in length of the galumnid mites.

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OVERWINTERING HABITS OF DOMESTIC FLIES AT CHARLESTON, WEST VIRGINIA

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During the winter periods of 1950–51 and 1951–52, studies on the overwintering habits of certain domestic flies were conducted at Charleston, West Virginia. Principal emphasis was placed on those species which composed the major portions of the fly population during the fly season. These species were *Phormia regina*, *Phaenicia sericata*, *Phaenicia caeruleiviridis*, *Phaenicia pallescens*, *Ophyra leucostoma*, and *Musca domestica*. In addition, observations were also made on the prevalence and activity of the cool weather blow flies of the genera *Calliphora* and *Cynomyopsis*.

METHODS

In the 1950–51 operations, five permanent sites which had been areas of heavy summer infestations were selected for weekly observation. Diligent search for fly eggs, larvae, or puparia was made at each inspection, the ground or medium being probed to depths of 18 to 24 inches. All live specimens were brought to the field office for attempted rearing and for identification purposes. In the 1951–52 winter, survey practices were altered from intensive coverage of selected stations to surveillance on an extensive, randomized basis. During the first winter, trap operations occurred whenever weather conditions appeared suitable for fly movement; in the second year, routine weekly trap sampling was established. In addition, observations were included on adult occurrence in both outdoor and indoor environments, and emergence cages were located over sites of copious late fall fly production.

In the 1950–51 winter period, weather conditions were unusually rigorous, characterized on several occasions by deep snows and subzero temperatures. Average minimum temperature and total snowfall for the period November to March were 25.8° F. and 39.8 inches, respectively. During 1951–52, weather conditions were relatively mild, the temperature never reaching subzero levels and with a minimum amount of snowfall. (Average minimum temperature 31.8° F., 9.3 inches snow.)

RESULTS

From the viewpoint of locating fly larvae and puparia, the weekly surveys of the 3 refuse dump areas, 1 landfill site, and a rubbish dump were productive, specimens being detected in all of the 69 inspections which were effected between November, 1950 and March, 1951. How-

ever, relatively few of the collected larvae or puparia succeeded in completing development to the adult stage. Therefore, many of the data are primarily qualitative and merely establish the fact that the immature stages of certain species were present in the described habitats during the winter period.

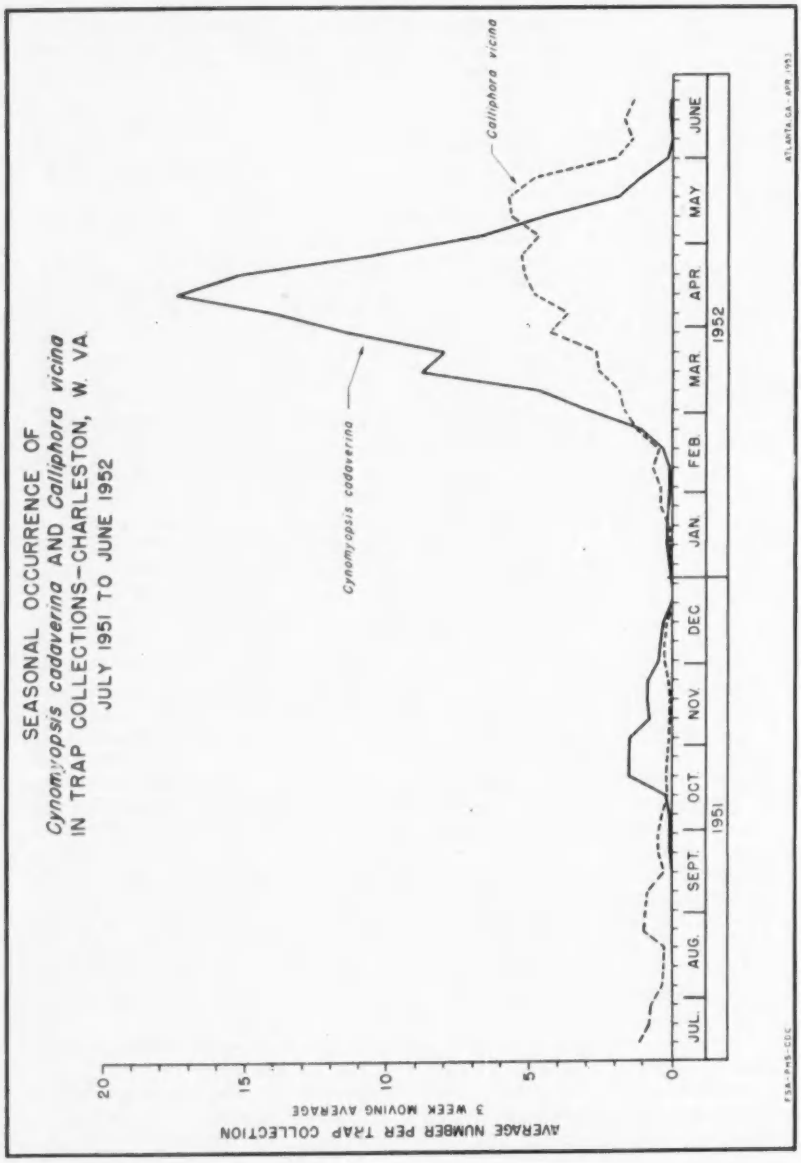
The majority of the positive collections came from garbage-soaked newspapers, garbage, or from a soil-garbage mixture. Numerous specimens were located in newspapers but their presence was disclosed only by carefully tearing apart the individual pages or sheets. Collections from the four dump areas yielded *Calliphora*, *Cynomyopsis cadaverina* (R. D.), *Phaenicia sericata* (Mg.), *Phaenicia pallescens*, (Shannon), *Phormia regina* (Mg.), *Stomoxys calcitrans* (L.), *Ophtra leucostoma* (Wd.), *Ophyra aenescens* (Wd.), and *Scopeuma stercorarium* (L.). Except for *O. aenescens* and *Calliphora*, only one or two specimens of each of these flies were obtained. *Calliphora* occurred chiefly in rat carcasses and was taken at all four sites. *Ophyra aenescens* was recovered from three stations, being extremely abundant at one refuse dump.

At the landfill site, specimen collections were made in an area of the fill which had been free of dumping operations for the previous 3 months. Fly larvae and puparia were recovered here more readily than at the dump sites. Although the landfill station provided the greatest variety in the types of flies produced, most of the specimens belonged to families of minor importance in community fly control (*e.g.*, Borboridae, Helomyzidae, Piophilidae, Sepsidae, Anthomyidae, Otitidae).

Phaenicia

Despite intensive effort to find the overwintering forms of these three blow fly species, larvae or puparia were rarely recovered. Extensive and systematic digging to depths of 24 inches in varied favorable locations failed to yield *Phaenicia* even though larvae of other calliphorid genera were obtained. Since other workers (Hagmann and Barber, 1948; Holdaway and Evans, 1930; Green, 1951a and b) report *P. sericata* to hibernate in the larval stage, it was anticipated that recovery of larvae by the present methods would not be too difficult. While the larvae could have escaped detection by descending to depths greater than 24 inches, Hagmann and Barber (1948), and Green (1951a and b), indicated that penetration of soil seldom exceeded 6 inches. The two positive samples obtained confirm this statement. A single larva of *P. sericata* was found at a subsurface level of 2 inches in a mixture of soil, paper, and garbage on February 12; on March 8, larvae of *P. pallescens* were discovered at a depth of 4 inches in burnt debris near a rat carcass.

Adult *P. sericata* occurred in numbers in May; *P. caeruleiviridis* in May–June; *P. pallescens* in June–July. All species were of frequent occurrence until early fall. Last appearance for the three species in 1951 was in trap samples procured in dump areas on December 7. Specimens of *P. sericata* first reappeared in trap collections in March, 1952, the earliest date being March 13; *P. caeruleiviridis* in May, and *P. pallescens* in April. Three observations in the January–February


SEASONAL OCCURRENCE OF
Cynomyopsis cadaverina AND Calliphora vicina
IN TRAP COLLECTIONS—CHARLESTON, W. VA.
JULY 1951 TO JUNE 1952
Calliphora vicina
Cynomyopsis cadaverina
AVERAGE NUMBER PER TRAP COLLECTION
3 WEEK MOVING AVERAGE
20
15
10
5
0
JUL.
AUG.
SEPT.
OCT.
NOV.
DEC
JAN.
FEB.
MAR
APR.
MAY
JUNE
1951
1952

period of *Phaenicia* on the wing presumably can be referred to *P. sericata* as this species was the one most commonly taken in cold weather.

Phormia regina (Meigen)

The black blow fly occurred throughout the winter. Adults were taken on the wing during warm days or recovered from open cellars or beneath porches in a torpid state during periods of freezing weather. These data support the general opinion (Hall, 1948; Roberts, 1930) that this species passes the winter as a semi-active adult. With the advent of spring, population densities gradually increased, reaching their maximum in the late May–June period.

Calliphora vicina Macquart

The bluebottle fly, *Calliphora vicina*, appeared in trap collections throughout 1951 and up to July 1952 with the exception of the December 9–January 12 period (figure 1). Peak captures of this species occurred between mid-March and early May with summer prevalence low. The absence of adults in midwinter[1] could represent the hibernating phase of this species as described by Hall (1948) and Green (1951b). The latter indicates the overwintering stage in England to be the larva whereas Holdaway and Smith (1932) in Australia report the pupa as the hibernating form. The occurrence of adult *C. vicina* on the wing in winter whenever warm weather prevailed suggests the possibility that some overwintering may occur as an imago, particularly during mild winters. In the latter instance, continuous retarded reproduction is possible since Green (1951b) reports adult emergence at air temperatures of 32° to 36° F.

A fly infestation of a dog carcass discovered on March 17, yielded *C. vicina* and *Cynomyopsis cadaverina*. Mature larvae and prepupae were recovered from the soil in cells 3 feet from the animal, chiefly in the roots of shrubs. Fully pigmented puparia occurred 3 to 6 feet away. Both larvae and puparia were located at depths less than 2 inches below the ground level. Temperature of the soil at the time of collection was 40° F. Removal of prepupae to room temperature resulted in adult emergence 10 days later.

Cynomyopsis cadaverina (Robineau-Desvoidy)

The shiny bluebottle fly is essentially a cool weather species, specimens rarely being trapped during the summer months (figure 1). In 1951, *C. cadaverina* appeared in early fall, persisted until mid-December, and reoccurred in January, reaching its maximum density in the spring.

[1]The collections for the December 9 to January 6 period were negative for all species of flies so the significance of the absence of *C. vicina* probably is less than it appears.

EXPLANATION OF FIG. 1

FIG. 1. Seasonal occurrence of *Cynomyopsis cadaverina* and *Calliphora vicina* in trap collections, Charleston, W. Va., July, 1951, to June, 1952.

The fall peak frequently is higher than the maximum level depicted for 1951–52; in the previous year, the trap index approached that of the spring peak period in 1952.

Based on trap collection data from December, 1951 through March, 1952, *C. cadaverina* was the most abundant and active fly in the Charleston area during that period (table I).

As with *Calliphora vicina*, the same situation concerning the identity of the hibernating stages and period concerned prevails. Roberts (1930) was unable to demonstrate adult survival at Ames, Iowa; however, the frequent observation of adults on the wing at Charleston immediately following the occurrence of warm winter days suggests that some survival takes place in this form.

One instance of recovery of larvae and puparia of *Cynomyopsis cadaverina* and *Calliphora vicina* was described under the latter species.

TABLE I

RELATIVE ABUNDANCE OF FLIES TRAPPED AT CHARLESTON, WEST VIRGINIA, NOVEMBER—MARCH, 1951–2.

SPECIES	PERCENT OF TOTAL
Cynomyopsis cadaverina	47.5
Calliphora vicina	19.0
Pollenia rudis	14.2
Phormia regina	5.3
Calliphora vomitoria	3.6

Pollenia rudis (Fabricius)

While the activities of *Pollenia rudis* did not receive much attention in these studies, the available information agreed with that previously reported by other workers. The species is active throughout the winter whenever temperature conditions are favorable. Trap collections from January 1 to July 1, 1952, indicate *P. rudis* to be on the wing during that period, maximum numbers being captured in late March. The parasitic habits of *P. rudis* larvae upon earthworms preclude detection of this species in the normal fly breeding substrates.

Musca domestica Linnaeus

The house fly was not a predominant species in the Charleston, West Virginia, area. The major period of activity occurred in late July and August, but adult flies persisted outdoors in the fall, particularly at favorable breeding sites. Large numbers were noted at one refuse dump on November 27, 1950. The same population was still present on December 4, but disappeared the following week. Except

for an occasional adult observed indoors in dairies or homes, these were the only house flies detected during the winter period of 1950.

In the winter of 1951–52, two observations indicated that house fly breeding does occur in heated areas where insanitary conditions prevail. On January 30, adult *M. domestica* were noted in a meat market and in the squalid basement of a pool hall. The latter harbored numerous cats whose accumulated excrement was serving as an excellent breeding medium for *M. domestica*.

The second winter fly infestation was detected in late January in a dirt-floored heated subterranean passway (150′ x 12′) which was being utilized for entry to several storage rooms and to two kitchens in which food was prepared for street level restaurants. Along the center of

FIG. 2. A fly overwintering area: battered, lidless, neglected garbage cans.

the passageway 10 to 12 receptacles were located for the storage of food wastes from the 2 kitchens. All of the garbage containers were in decrepit condition (figure 2). Battered and without lids, the receptacles contained a bottom layer of organic sludge despite daily removal of garbage. In each can, the sludge was heavily infested with *M. domestica* larvae. In addition, house fly larvae were also recovered from cat excrement. Needless to state, the adjoining kitchens were alive with adult *M. domestica*.

From these instances of active breeding and the frequent observation of adults in heated buildings, it is apparent that *M. domestica* can overwinter in insanitary indoor situations in numbers which appear wholly adequate to maintain the population until the advent of suitable outdoor breeding conditions. In the literature, the overwintering habits of this common fly have been the subject

of much controversy, various authors favoring one or more stages as the means by which the species perpetuates itself through periods of low temperatures. As West (1951) indicates, the conflicting reports arise in part from the fallacy of comparing fly activity in climates which are markedly different. Another factor has been the emphasis upon outdoor habitats of the species with little attention being paid to certain situations wherein prevailing temperatures permit the survival of the species. Under this category would fall habitats subject to artificial heating or to the higher temperatures caused by biological fermentation and the burning of combustible wastes mixed with organic refuse. Matthysse (1945) reported continuous breeding of *M. domestica* in dairy barns and heated buildings in northern New York and considered indoor propagation of the species as its most important method of overwintering. Hewitt (1915) stressed the presence of adult flies in warm bakeries, kitchens, restaurants and stables. Although Hewitt indicated these adults to be fertile, he did not report any observations of fly breeding. Dump areas where burning and fermentation occur also produce temperatures suitable to fly breeding (see *O. aenescens*). Thus the writers feel that the continuous breeding phase of overwintering merits greater attention in studies of the survival of *M. demestica* in northern climates.

This emphasis on indoor continous breeding does not preclude outdoor survival as hibernating larvae or puparia or even as the imago. With an insect as versatile and as adaptable in its behavior under changing environments as the house fly, it is entirely reasonable to expect that, faced with adverse conditions for survival, the species could and would react with more than one type of response.

Ophyra

Both *Ophyra leucostoma* and *O. aenescens* were present in the Charleston, West Virginia, area. The former was predominant, ranking second in abundance of the total flies collected on citywide trap surveys during the May through October period. *O. aenescens* was trapped only infrequently. However, despite the widespread occurrence of *O. leucostoma* in the community, little information was gleaned on its winter activity. Larvae of *O. leucostoma* were obtained on February 19 at a refuse dump and from blood-soaked soil at a local slaughterhouse on February 28.

In contrast, sampling sites at one of the refuse dumping areas proved to be a continuous source of *O. aenescens*. Both larvae and adults were observed throughout the period December 6, 1950 through March 15, 1951. With air temperatures of 39° to 56° F., adults moved actively either on or just below the surface of the dump. On December 28 (temperature 46° F.), a grill count using an 18-inch square grid disclosed approximately 120 specimens, all *O. aenescens*. At all times, larvae of this species could be found by tearing apart garbage-soaked sheets of newspapers or other debris.

Careful observation of this dump area for 2 years has revealed that only *O. aenescens*, never *O. leucostoma*, is produced there. Even during the summer, the population remains the same despite the general prevalence of *O. leucostoma* in the community.

Since *O. aenescens* is found more frequently in warmer climates and *O. leucostoma* in the northern areas, the year-round breeding of *O. aenescens* at this dump approaches an enigma unless consideration is given to temperature levels which prevail within the dump habitat. Studies at this dump reveal that the fermentation processes and refuse burning practices provide subsurface temperatures much higher than those of the surrounding air. The temperature differential is contingent upon the degree of fermentation in the medium, the amount of refuse burning, and the depth of the sample. During the 1950–51 winter, habitat temperatures never dropped below 40° F. despite air temperatures as low as 16° to 33° F. (table II). It is concluded that the consistently higher habitat temperature was the factor responsible for the year-round breeding of *O. aenescens*.[2]

TABLE II

AIR AND HABITAT TEMPERATURES (Degrees Fahrenheit) AT THE REFUSE DUMP, DUNBAR, WEST VIRGINIA, 1950–51.

	Sample Depth Inches	Medium	Habitat Temp.	Air Temp.
Dec. 15	1–2	Garbage	59	42
20	2–3	Compost animal carcass	51	40
Jan. 4	1	Garbage	50	42
18	2	"	74	58
31	3	"	46	33
Feb. 2	2	"	88	16
13	3	"	55	64
Mar. 1	1–3	"	65	62

As to why *O. aenescens* can maintain itself in "pure culture" in a zone dominated by *O. leucostoma* is an enigma. Dr. H. R. Dodge[3] has observed two other localized infestations of this nature at Sheffield, Alabama, and Caldwell, Idaho, but neither of these has been satisfactorily explained. Possibly *O. leucostoma* is unable to withstand the competitive presence of *O. aenescens*.

SUMMARY

1. Studies on the overwintering habits of *Phaenicia sericata*, *P. caeruleiviridis*, *P. pallescens*, *Musca domestica*, *Phormia regina*, *Ophyra*, *Calliphora vicina*, and *Cynomyopsis cadaverina* were conducted at Charleston, West Virginia, during 1950–51 and 1951–52.

2. Breeding of *M. domestica* was demonstrated in cat excrement and garbage sludge present in basements of heated buildings during

[2]The same dump also serves a year-round breeding source for the cockroach *Blattella germanica* and the earwig *Euborellia annulipes*.

[3]Personal communication.

the midwinter period. Data indicate that the continuous breeding phase of house fly overwintering merits greater attention in studies of survival of this species in northern areas.

3. *Cynomyopsis cadaverina* was the predominant fly in the winter trap collections, constituting 47.2 percent of the 2127 specimens recovered. Other species were: *Calliphora vicina* (19 percent), *Pollenia rudis* (14.2 percent), and *Phormia regina* (5.3 percent).

4. The occurrence of *Calliphora vicina* and *Cynomyopsis cadaverina* on the wing whenever warm weather prevailed indicated that these species probably overwinter as adults. The period of peak prevalence for each species was in early spring.

5. Continuous breeding of *Ophyra aenescens* throughout the winter occurred in one dump area. Higher habitat temperatures were considered to be the factor responsible for this propagation.

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THE NERVOUS SYSTEM OF THE PREGENITAL ABDOMINAL SEGMENTS OF SOME ORTHOPTERA[1]

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The general plan of the arrangement of the muscles of the abdomen has been well established (Snodgrass 1935a). The system of nerves reaching these muscles should, it would seem logical, also exhibit a basic plan, if criteria of homology of nerves can be established. This study was undertaken to determine if such criteria exist in the limited field of the pregenital abdominal segments of some typical orthopterous insects. In the course of the work some details were discovered on the innervation of the ventral diaphragm which are included as a second section of this paper.

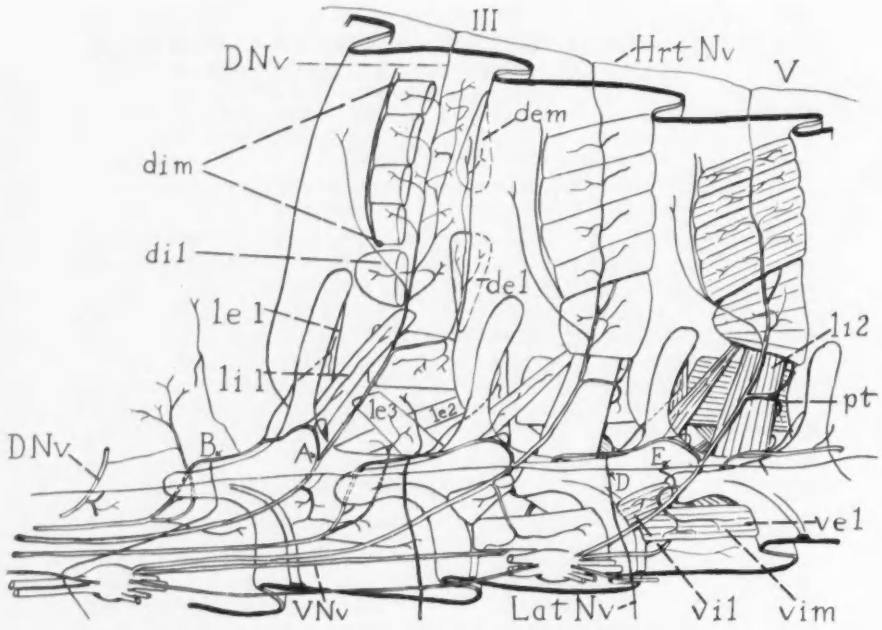
1. THE PLAN OF THE SEGMENTAL SYSTEM

The Carolina Locust, Dissosteira carolina

The abdominal musculature of the Carolina locust, *Dissosteira carolina*, has been described in excellent detail by Snodgrass (1935b). This species was therefore used as the starting point of this investigation. The general arrangement of the nerves of the right half of abdominal segments 3, 4 and 5 is shown in Plate I. Two pairs of large nerves may be seen to arise laterally from each segmental ganglion. One nerve of each pair provides the innervation of the dorsal group of muscles and fuses with the heart nerve. Thus the designation "dorsal nerve" (DNv) seems to be reasonably appropriate. The second pair of nerves, as seen in *Dissosteira*, provides the innervation of one of the ventral muscles and of three of the lateral muscles within the segment of its origin. It does not terminate at this point, but continues posteriorly into the succeeding segment and there becomes a branch of the dorsal nerve of that segment. This branch also supplies the innervation of the anterior lateral muscles of that segment and of the spiracular valve. However, this second nerve from each ganglion provides also a branch which passes to the ventral body wall in all the Orthoptera examined. This second nerve is therefore called the "ventral nerve", although its exact limits cannot at present be defined.

These details of the arrangement of the muscle innervations are shown in diagramatic form in figure 1, Plate II. Each individual muscle is indicated by an underlined abbreviation. Points at which nerves pass but are not joined are represented by an interruption of the more laterad nerve, while nerve junctions are represented by the absence of such an interruption. The connectives between the seg-

[1]Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Entomology.



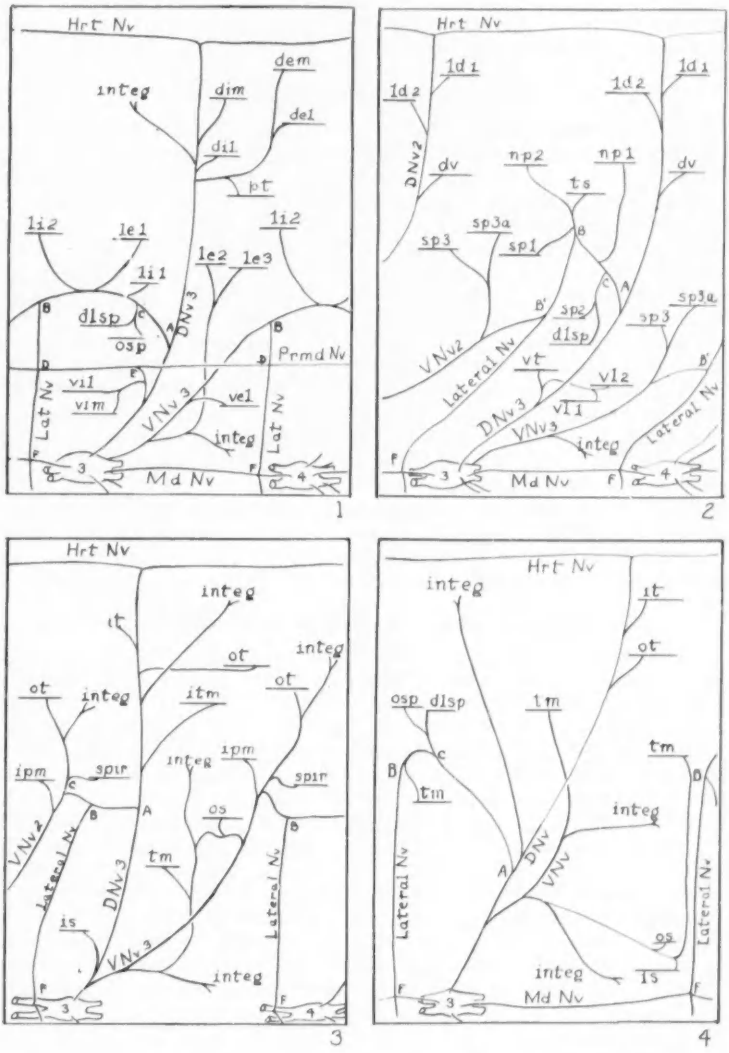
Dissosteira carolina. Muscles of the right side of abdominal segments three, four and five, together with the nerves of those segments.

LIST OF ABBREVIATIONS

(NOTE: The names applied to individual muscles are taken directly from the literature cited on each insect as described in the text of this paper.)

del—dorsal external lateral muscle
dem—dorsal external median muscle
dil—dorsal internal lateral muscle
dim—dorsal internal median muscle
dlsp—dilator of the spiracle
DNv—dorsal nerve
DSl Nv—dorsal nerve
dv—dorsoventral muscle
Hrt Nv—heart nerve
integ—integument
ipm—intersegmental pleural muscle
is—intersternal muscle
it—intertergal muscle
itm—intersegmental tergo-sternal muscle
Lat Nv—lateral nerve
ld 1—first dorsal muscle
ld 2—second dorsal muscle
le 1—first external lateral muscle
le 2—second external lateral muscle
le 3—third external lateral muscle
li 1—first internal lateral muscle
li 2—second internal lateral muscle
Md Nv—median nerve
np 1—fourth lateral muscle
np 2—fifth lateral muscle
os—outer sternal muscle
osp—occlusor of the spiracle
ot—outer tergal muscle
pt—paratergal muscle
Prmd Nv—paramedian nerve
sp 1—first lateral muscle
sp 2—second lateral muscle
sp 3—third lateral muscle
sp 3a—third (a) lateral muscle
Spir—spiracular muscle
tm—tergo-sternal muscle
vel—ventral external lateral muscle
vil—ventral internal lateral muscle
vim—ventral internal median muscle
vl_1—first ventral muscle
vl_2—second ventral muscle
VNv—ventral nerve
vt—transverse ventral muscle

(Upper case letters A–F indicate points used as criteria of homology.)



Diagrams showing the nerve patterns of the right side of a typical pregenital abdominal segment, viewed mesally. FIG. 1, *Dissosteira carolina*. FIG. 2, *Acheta assimilis*. FIG. 3, *Periplaneta americana*. FIG. 4, *Diapheromera femorata*.

mental ganglia are represented as severed at each ganglion in order to show more clearly the unpaired median nerve (Md Nv) and the lateral nerves (Lat Nv). These lateral nerves join the ventral nerve at the point "B", providing a "landmark" of considerable usefulness.

The Cricket, **Acheta assimilis**

The abdominal musculature of the cricket has been described by DuPorte (1920) and his descriptions are used in this discussion. The nervous system of the right side of the third abdominal segment is shown diagramatically in figure 2, Plate II. The same conventions are used as in figure 1.

The ventral musculature of *Acheta* consists of four longitudinal muscles and a single transverse muscle. The innervation of the longitudinal muscles, vl_1 and vl_2, by a branch from the dorsal nerve indicates that these muscles belong to the same series as the internal ventrals, *vil* and *vim*, of *Dissosteira*. The transverse muscle, *vt*, receives a nerve at each end, from the same branch of the dorsal nerve.

The ventral nerve in *Acheta* joins the lateral nerve at B^1, and the resulting nerve, to B, appears to be a fusion of the two. The innervation of the dilator of the spiracle, *dlsp*, appears to come from the dorsal nerve, at C. The sternopleural muscles receive innervation from the loop of the ventral nerve and the branch of the following dorsal nerve at A, in much the same manner as the lateral muscles in *Dissosteira*. Apparently it will be necessary to trace the individual axones in order to determine the ganglionic source of the innervation of the lateral and the spiracular muscles.

The American Cockroach, **Periplaneta americana**

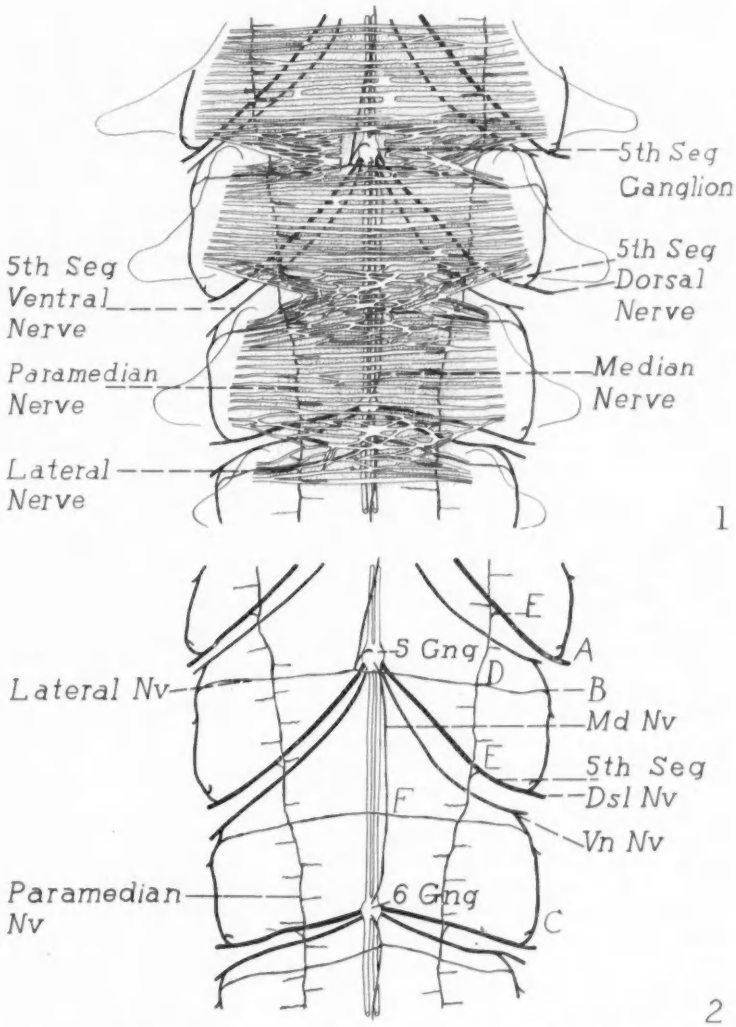
The work of Ford (1923) was used as a guide to the abdominal musculature of *Periplaneta*. The nerve plan of the right side of a segment is shown diagramatically in figure 3, Plate II. The unpaired median nerve of *Periplaneta* has fused with the intersegmental connective, as shown by the apparent origin of the lateral nerves from the right intersegmental connective. A somewhat similar condition has been observed in a few specimens of *Acheta*, in which the median nerve adhered to the connective for part of the interganglionic distance.

The dorsal nerve and the ventral nerve emerge from the ganglion as a single root, but separate just beyond the ganglion. There is only a single internal ventral muscle, *ls*, innervated by a branch from the dorsal nerve, as in *Dissosteira* and *Acheta*. The innervation of the outer sternal muscle, *os*, although provided by the ventral nerve as is the ventral muscle of *Dissosteira*, is more complex, involving a fusion of branches from the two primary branches of the ventral nerve.

The innervation of the spiracular valve muscle at C also differs from *Dissosteira*, although we again need to know the precise route of the individual axones to evaluate this difference.

The Northern Walkingstick, **Diapheromera femorata**

The description of the abdominal musculature of the Phasmidae provided by Ford (1923) was used as a guide. The general plan of



Dissosteira carolina. Innervation of the ventral diaphragm. FIG. 1. The ventral diaphragm and its associated nerves in abdominal segments four, five and six as viewed dorsally. FIG. 2. The nerves associated with the ventral diaphragm in segments four, five and six.

See page 678 for abbreviations.

the nervous system of the right side of a typical segment is shown in figure 4, Plate II.

In this insect, the median nerve resembles that of *Dissosteira*, and provides the usual lateral branches. The main nerves from each side of the ganglion are fused for a short distance, but branch to establish the dorsal nerve, *DNv* and the ventral nerve *VNv*. The dorsal nerve does not differ particularly from the form seen in other groups, although no innervation of the sternal muscles is provided by it. The spiracular valve muscles receive innervation near the point B, where the lateral nerve joins the branch from the dorsal nerve originating at A. There was no trace found of any extension of the ventral nerve of the preceding segment into the nerve complex associated with the spiracular valve muscles, as in the cases described thus far.

The ventral nerve in the walkingstick provides the only innervation of the sternal muscles, and in addition it enters the tergal-sternal muscle group, *tm*, through two primary branches. This muscle group, which extends the full length of the segment, is also innervated at B, and thus appears to belong to the same muscle group as the lateral muscles of *Dissosteira*.

2. THE INNERVATION OF THE VENTRAL DIAPHRAGM

The ventral diaphragm in *Dissosteira* is illustrated in figure 1, Plate III. It is possible to distinguish an extremely fine pair of nerves extending laterally along the full length of the diaphragm. These nerves, here called the ***paramedian nerves***, *Prmd Nv*, provide branches to the fibers of ventral diaphragm. In figure 2, Plate III, this system is illustrated as it would appear if the diaphragm could be removed.

Each paramedian nerve is connected to the nervous system at two points in each segment. The first of these involves the lateral nerve at D, and the second takes place by way of the branch of the dorsal nerve which provides innervation of the ventral internal muscles (figure 1, Plate II) at E. No trace of the paramedian nerve was found in the groups examined which lacked the ventral diaphragm.

A system of nerves closely resembling the paramedian is described by Maki (1936) as occurring in a neuropterous insect, *Chauliodes formosanus* Petersen. Maki described the part anterior to the lateral nerve as an anterior branch of the lateral nerve, and described the posterior part as a posterior branch of the lateral, although the parts are continuous. He made no reference to an innervation of the ventral diaphragm.

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THE LARVAL AND PUPAL DEVELOPMENT OF THE HONEY BEE APIS MELLIFERA LINNAEUS[1]

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During the past one hundred years the honey bee, *Apis mellifera* L., has been the object of a vast amount of research. The physiology and behavior of the honey bee are being investigated at the present time by various agencies interested in the animal as a food producer and pollinator. The anatomy, embryology, and morphology have already been the object of considerable study by entomologists.

More recently metamorphosis in insects has become an area of intensive research. This paper deals primarily with metamorphosis since it is concerned with the larval and pupal development of the honey bee. According to Snodgrass (1925), "metamorphosis is practically limited to that between the last larval stage and the imago." He also defines metamorphosis "as any change of form or structure that an animal undergoes which is not in line with direct development, or which results from a deviation from it." After close observation of the day to day development in the honey bee from time of hatching through pupation, it becomes not only difficult, but practically impossible, to determine any change of form or structure not in line with direct development.

This report describes the external changes in structure and form as they occur from day of hatching through nine days after hatching (early pupation). Drawings were made of the larvae, prepupae, and pupae which indicate the gradual changes undergone during this interval.

PART I

EXTERNAL STRUCTURAL CHANGES DURING LARVAL AND PUPAL DEVELOPMENT

METHODS AND MATERIALS

Procurement of bees. This problem was started late in the fall of 1950. In order to obtain a continuous supply of living material three hives of bees were placed in the greenhouse and were kept at a temperature of 75° F. to 80° F. A method of marking capped cells was devised which enabled the writer to secure late larvae and pupae of a

[1]This research was done in partial fulfillment of the requirements for the Doctor of Philosophy degree, the department of Zoology and Entomology, The Ohio State University, Columbus, Ohio.

[2]The author wishes to express his sincere appreciation to the late Dr. C. H. Kennedy for his direction and kindly criticism of this research; to Dr. W. E. Dunham, Mr. Charles Reese, and Mr. John Knierim for supplying the bees, hives, honey, and pollen used in this study and for other technical advice given during this research.

known age. In this research, as in Oertel's (1930), specimens were gathered at twenty-four hour intervals, or oftener, throughout pupation. A brood frame, containing eggs, larvae, and capped brood, was removed from a hive. All of the capped cells in the frame were daubed with nail polish and the frame was then replaced in the hive. Twenty-four hours later the same brood frame was removed and all the cells having been capped during this interval were daubed with nail polish of a different color from that used on the previously marked capped cells. By using nail polish of different colors, each day's group of capped cells could be marked on the brood frame. Thus a larva, prepupa, or pupa of a relatively known age could be removed from the brood frame whenever suitable to the investigator.

EXPLANATION OF PLATE I

FIG. 1. Lateral view of a day-old larva. FIG. 2. Lateral view of an early two day-old larva. FIG. 3. Lateral view of a late two day-old larva. FIG. 4. Lateral view of an early three day-old larva. Fig. 5. Lateral view of a medium three day-old larva. FIG. 6. Lateral view of a late three day-old larva. FIG. 7. Lateral view of a four day-old larva. FIG. 8. Lateral view of a full grown five day-old larve or "day of capping" larva. FIG. 9. Lateral view of a six day-old larva or "one day after capping" larva (early prepupa). FIG. 10. Lateral view of a seven day-old larva or "two days after capping" larva (prepupa).

ABBREVIATIONS USED IN THE FIGURES

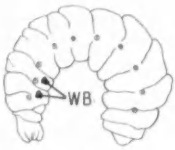
A.....antenna
ABD.....abdomen
AN.....anus
AO.....aorta
BR.....brain
CA.....corpora allata
CLP.....clypeus
E.....eye
FB.....fat bodies
1G.....
2G.....gonaphophyses
3G
FG.....frontal ganglion
F.GUT....foregut
FN.....frontal nerve
H.....head
HT.....heart
LAB.....labium
LA.P .....labial palpi
LB.....leg bud
LBR.....labrum
LCT.....lancet
LS.....larval skin (cuticle)
L1
L2.....legs
L3
M.....muscle
MD.....mandibles
MPS.....mouthparts
M.GUT....midgut
MX.....maxilla
MX.P.....maxillary palpi
O.....oesophagus
OEN.....oenocytes
OES.G.....oesophageal ganglion
OP.L.....optic lobes
PS.....pupal skin (cuticle)
P.TH.....prothorax
RN.....recurrent nerve
SAL.D.....salivary duct
SD.....silk duct
SH.....sheath
SOG.....suboesophageal ganglion
SP.....spiracle
ST.P.....sting palpi
T.....trachea
TEN.....tentorium
TEN.A....tentorial arm
TH.....thorax
TH.G.....thoracic ganglion
VNC.....ventral nerve cord
W1
W2.....wings
W3
WB.....wing buds

EXPLANATION OF PLATES

All of the drawings showing the external structures in the honey bee (Plates I through VII) were made with the aid of a camera lucida. Figures 1 through 18 were drawn with a camera lucida and binocular microscope equipped with a 9X ocular and a IX objective.

PLATE I

1



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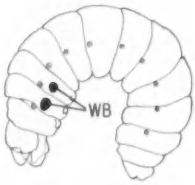

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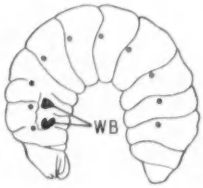

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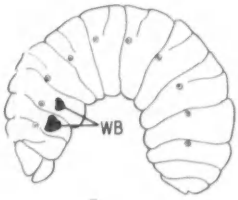

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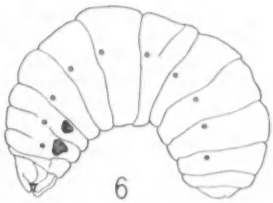

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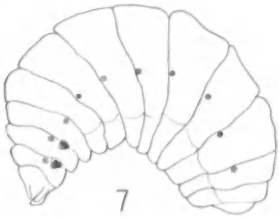

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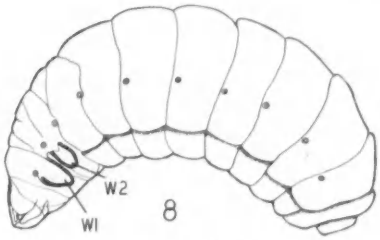

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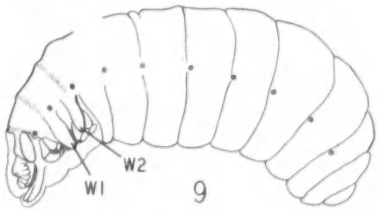

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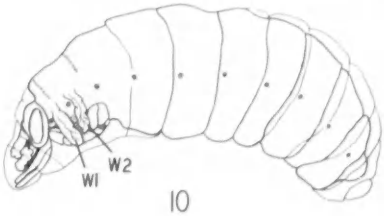

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A slight variation of this procedure was carried out by using a two-frame observation hive. This hive was placed on a small table in the middle of a greenhouse cubicle. The two brood frames fitted in the hive one above the other. Both sides of this hive were glass which afforded a good view of the bees at any time. One side of the hive was hung with two glass doors, which, when opened, provided easy access to the bees and to the brood. With this type of hive it was possible to remove one of the frames without disturbing the other. In some instances the brood was removed from the side of the frame next to the glass doors without removing the frame or causing undue disturbance to the worker bees in the area. The time of capping of the brood cells by the worker bees was easily noted and the capped cells were immediately marked with nail polish.

The use of nail polish daubed on the capped cells proved satisfactory because: (1) It dried quickly; (2) the attendant worker bees did not chew it off and thus the cells retained the characteristic mark during the whole interval of pupation; (3) the polish and its solvents did not kill or in any way appear to affect the development of the individual within the capped cell.

One of the early difficulties encountered in this study was securing sufficient brood of a known age. Many times actual counts were made of eggs laid in the cells of a brood frame. Upon later examination of the same brood cells the writer found many, if not all, of the eggs gone. This disappearance was accounted for when a worker bee was observed eating the eggs (Myser, 1952).

Killing and fixing the young bees. The ends or caps of the cells were pierced and peeled back exposing the larvae, prepupae, or pupae which could then be removed from the brood cells with a pair of fine-pointed forceps by careful pulling. They were then dropped into vials containing K.A.A.D.

The K.A.A.D. solution used was developed by Dr. Alvah Peterson (1948) of the department of Zoology and Entomology of The Ohio State University. It is a mixture of one part kerosene, seven to ten parts 95% alcohol, two parts glacial acetic acid, and one part dioxane. K.A.A.D. has proven to be an excellent killing solution because it quickly penetrates the cuticle and the larvae expand. Once fixed in K.A.A.D. the material did not shrink or distort when stored or dehydrated in alcohol. Twenty-four hours after killing, the immature bees were transferred to 70% alcohol and stored until needed for dissection or for making illustrations. About 350 bees in various

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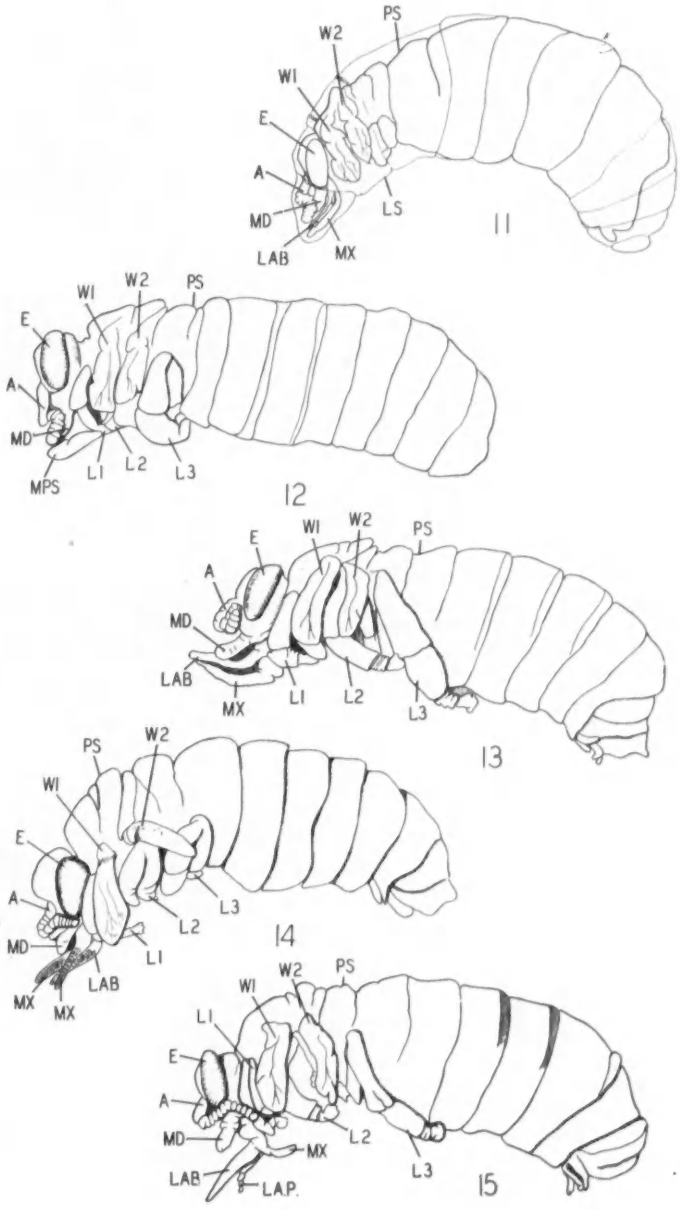
EXPLANATION OF PLATE II

FIG. 11. Lateral view of an eight day-old larva or "three days after capping" larva (late prepupa).
Fig. 12. Lateral view of an early pupa.
FIG. 13. Lateral view of an early pupa.
FIG. 14. Lateral view of an early pupa.
FIG. 15. Lateral view of an early pupa.

These drawings show the appendages of the head and thorax in the various stages of unfolding and becoming adult-like.

PLATE II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stages of development were obtained from the brood frames of the hives and prepared in the above manner. Some of the bees of each stage were later sectioned and stained with iron hematoxylin in order to reveal the histological changes undergone during pupation.

LARVAL DEVELOPMENT

The one day-old larva. Certain conspicuous imaginal tissues can be located in the one day old larva and their development can be followed from day to day in the bee until their adult form is attained.

Beneath the larval cuticle on the lateral surface of the thorax are small, white crescent-shaped spots of tissue, the imaginal wing buds (Pl. I, FIG. 1, WB; Pl. V, FIG. 1B).

In the same stage three pairs of white disc-shaped spots of tissue are visible on the ventral surface of the thoracic segments, beneath the larval cuticle, which are the imaginal leg buds (Pl. VI, FIG. 1A).

Nelson (1924) has described the characteristics of the larval head.

> "The head may be described as bluntly conical, the base of the cone being formed by the head capsule, the apex by the labrum and other mouthparts. The ventral third of the head, including the labium, maxillae and the part of the head capsule to which they are attached, is sharply separated from the head capsule by a deep furrow which extends on each side from between the base of the mandibles and maxillae to the posterior limits of the head." (Pl. V, Fig. 1B; Pl. VI, Fig. 1A).

The clypeus and labrum are distinguishable only as a single projection from the mid-line of the anterior part of the head capsule between the antennal rudiments. Its distal end is free from the head capsule and is directed cephalad and ventrad (Pl. V, FIGS. 1B, 2B).

The mandibles originate from the head capsule as conical-shaped organs ventral to the antennal rudiments and on either side of the clypeus-labrum flap. They lie immediately dorsal to the maxillae and extend further cephalad.

EXPLANATION OF PLATE III

FIG. 16. Lateral view of an early pupa with the mouthparts extending posteriorly.

FIG. 16A. Ventral view of the head and thorax of an early pupa. The legs are still in close contact with the body and the skin is very wrinkled.

FIG. 17. Lateral view of a late "three days after capping" pupa. At this stage the adult form is nearly attained.

FIG. 17A. Ventral view of the pupa.

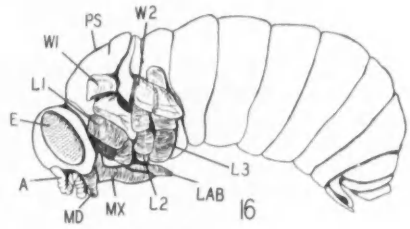
FIG. 18. Lateral view of an early "four days after capping" pupa. The pupal cuticle has loosened from the cuticle of the imago. All of the appendages are encased in this thin pupal cuticle.

FIG. 18C. Ventral view of the last four abdominal segments showing the appearance of the parts of the stinger. This drawing was made with the aid of a camera lucida and a binocular microscope equipped with a 15X ocular and a 1X objective.

FIG. 18D. Lateral view of the abdomen of a pupa showing the parts of the stinger. This drawing was made with the aid of a camera lucida and a binocular microscope equipped with a 15X ocular and a IX objective.

FIG. 19. Lateral view of a late "four days after capping" pupa.

PLATE III



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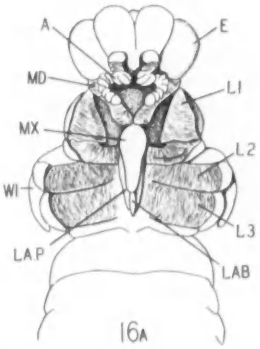

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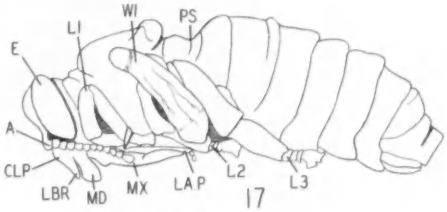

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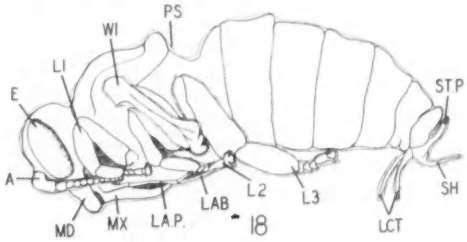

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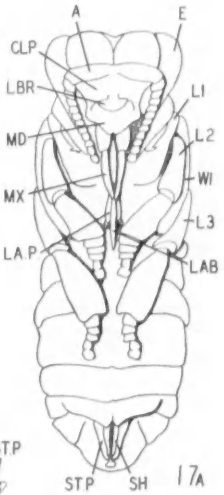

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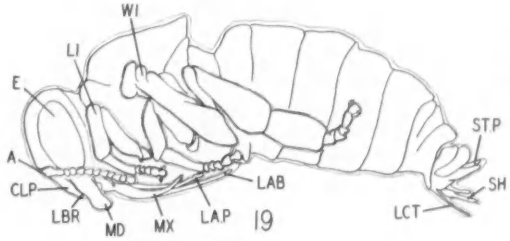

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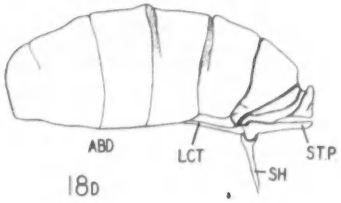

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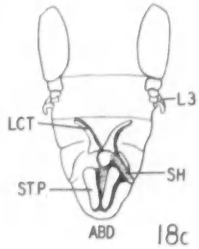

18C

The antennal rudiments can be seen in lateral view as rounded elevations on the head capsule above the base of the mandibles (Pl. V, FIG. 1B).

The labium can best be seen from a ventral view. In the one day-old larva it appears to originate from the mid-ventral line of the anterior third of the head and extends cephalad as a spatula-like lip. The maxillae can be seen lying laterad to the labium and also projecting cephalad (Pl. VI, FIG. 1A; Pl. V, FIG. 1B).

The two day-old larva. In the early two-day-old larva the wing buds and leg buds are more prominent and easier to see beneath the larval cuticle. In the region of the eighth and ninth abdominal segments on the mid-ventral side, white, fleshy rudiments or gonapophyses have now appeared (Pl. V, FIG. 2B; Pl. VI, FIGS. 2A, 2C).

The three day, four day, five day-old larva. From the third through the fifth day of development the major changes which occur are (1) an increase in the size of the thorax and abdomen; (2) an increase in the size of the hypodermal pockets and the leg buds, wing buds and gonapophyses within; (3) a proportionately smaller increase in the size of the larval head, but with the maxillae, labium, and mandibles becoming longer and thinner (Pl. V, FIG. 3B; Pl. VI, FIGS. 3A, 3C; Pl. V, FIGS. 4B, 5B, 6B, 7B, 8B; Pl.VI, FIGS. 4A, 4C, 5A, 5C, 6A, 6C; Pl. VII, FIGS. 7A, 7C, 8A, 8C).

The six day-old larva (one day after capping), early prepupa. In the region of the head and thorax the transparent larval cuticle is beginning to loosen from the pupal cuticle. The antennae can be seen plainly for the first time as S-shaped segmented organs on the front of the head.

The labium is now a slender, finger-like projection on the mid-ventral line of the head, bounded laterally by the two labial palpi which also extend cephalad. Dorso-lateral to the labial palpi are the two maxillae which extend cephalad (Pl. V, FIG. 9B; Pl. VII, FIG .9A).

The imaginal eyes are developing simultaneously on the lateral surfaces of the head as oval shaped structures (Pl. V, FIG. 9B).

The wing pads and legs are developing free of the hypodermal pockets. The wings are longer and extend farther down on the ventral surface of the body partially covering the legs on the mesothorax and metathorax. There are parallel transparent canals running from the base to the tips of the wings with an occasional branching canal

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EXPLANATION OF PLATE IV

Figures 11A, 11B, and 11D were all drawn with the aid of a camera lucida and binocular microscope equipped with a 12.5X ocular and 1X objective. Figures 11C, 20B, and 20D were drawn with a 12.5X ocular and a 2X objective.

FIG. 11A. Ventral view of the head and thorax of a late "three days after capping" larva (prepupa).

FIG. 11B. Lateral view of the head and thorax of a prepupa.

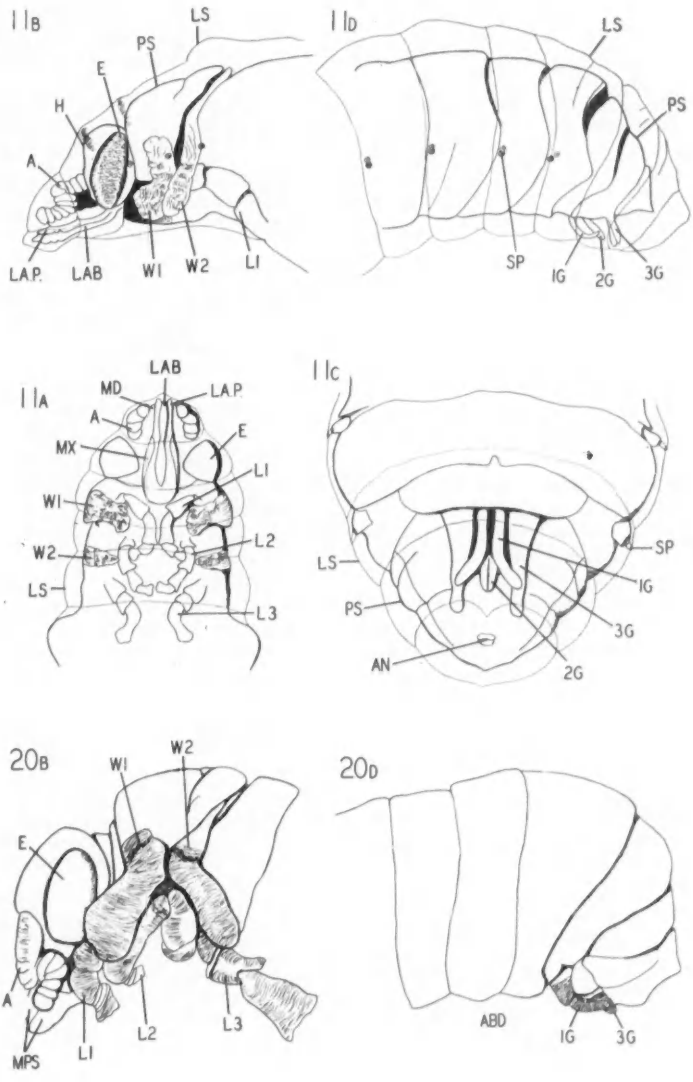
Fig. 11C. Ventral view of the gonapophyses.

FIG. 11D. Lateral view of the abdomen showing the gonapophyses and the pupa within the larval cuticle.

FIG. 20B. Lateral view of the head and thorax of an early "three days after capping" pupa. Note the wrinkled appearance of the wings and legs.

FIG. 20D. Lateral view of the abdomen of an early pupa.

PLATE IV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extending off at an oblique angle from the long canals. This is the first evidence of wing venation (Pl. V, FIG. 9B; Pl. VII, FIG. 9A).

The legs are now about three times longer than in the "day of capping" larva and the segments are distinguishable. The segments of the legs are folded in such a way that the tarsi cover the other segments ventrally and extend mesad where they meet on the mid-line of the thorax (Pl. VII, FIG. 9A).

The gonapophyses are now longer. The first pair extend caudad across the ninth abdominal segment then curve sharply at right angles and extend laterad about the same distance. The second pair have become longer, more slender and curve outward or laterad at their tips. The third pair are slightly broader at their bases than at their tips and extend to the same line posteriorly as the second pair (Pl. VII, FIG. 9C).

The seven day-old larva (two days after capping). The larval cuticle has loosened from the pupal cuticle of the head and thorax and also of the last four abdominal segments (Pl. I, FIG. 10). The wings are longer and the venation is more distinct, the mouthparts are longer, the legs are longer, the head is broader, the antennae and eyes are more prominent, and the gonapophyses are slightly longer than in the previous "one day after capping" larva (Pl. V, FIG. 10B; Pl. VII, FIGS. 10A, 10C).

The eight day-old larva (three days after capping), late prepupa. This is the climax of larval life. Now the larval cuticle is completely free from the pupal cuticle, and separated from it by moulting fluid, except for tracheal connections between the larval and pupal spiracles (Pl. IV, FIG. 11C). The head is about two thirds the adult size. The eyes are larger and their surfaces are creased and wrinkled. The antennae are still S-shaped but are longer and distinctly twelve segmented. The thorax is separating from the head by a distinct constriction. The wings are broader and more wrinkled than at any stage thus far and appear to be adhering closely to the thorax. The mouthparts are longer

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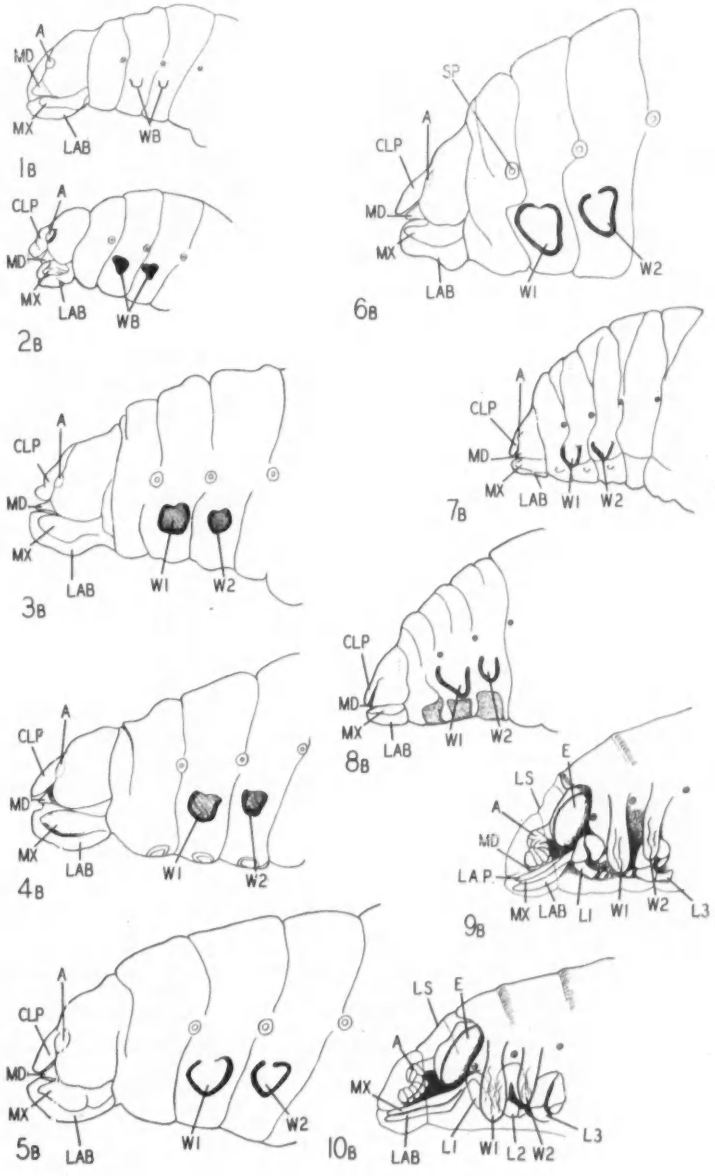
EXPLANATION OF PLATE V

Figures 1B through 6B were drawn with a binocular microscope equipped with a 9X ocular and a 3X objective. Figures 7B through 10B were drawn with a binocular microscope using a 12.5X ocular and a 1X objective. All of the figures were drawn with the aid of a camera lucida. Figures 1B through 10B correspond to Figures 1 through 10, Plate 1.

FIG. 1B. Lateral view of the head and thorax of a one day-old larva.
FIG. 2B. Lateral view of the head and thorax of an early two day-old larva.
Fig. 3B. Lateral view of the head and thorax of a late two day-old larva.
FIG. 4B. Lateral view of the head and thorax of an early three day-old larva.
FIG. 5B. Lateral view of the head and thorax of a medium three day-old larva.
FIG. 6B. Lateral view of the head and thorax of a late three day-old larva.
Fig. 7B. Lateral view of the head and thorax of a four day-old larva.
FIG. 8B. Lateral view of the head and thorax of a five day-old larva or a "day of capping" larva.
Fig. 9B. Lateral view of the head and thorax of a six day-old larva or a "one day after capping" larva (early prepupa).
FIG. 10B. Lateral view of the head and thorax of a seven day-old larva or a "two days after capping" larva (prepupa).

PLATE V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10B

and are fused into a compact group of cephalad projections (Pl. IV, FIGS. 11A, 11B).

The gonapophyses have changed position and are now fairly close to their adult relationship. The first pair of gonapophyses extends caudad and then laterad at a 45° angle. They lie ventral to, and cover the second pair which seem to have fused at their median surfaces. The third pair are broadly attached and have become longer, now extending farther caudad than the first or second pairs. The first pair will form the lancets of the stinger in the pupa, the second pair the sheath, and the third pair the sting palpi (Pl. IV, FIGS. 11C, 11D).

This stage of larval development is called the "prepupa" by Dobrovsky (1950), Snodgrass (1925) and others.

PUPATION

On December 5, 1951, the writer was examining the cells of a brood frame for prepupae and came upon some individuals which had just completed their last larval moult. According to Bertholf (1925) this is the beginning of pupation. The bodies of the pupae were still wet with moulting fluid. The bees were removed from the cells and examined under the binocular microscope for details.

The whole body surface or cuticle was covered with wrinkles but was not shrunken. The wings and legs, mouthparts and antennae, and gonapophyses appeared as flat, folded, deflated, wrinkled sacs adhering tightly to the thorax, head, and abdomen respectively. The head and thorax were narrower than the abdomen and the head was separated from the thorax by a distinct constriction. It was particularly difficult at this time to distinguish the individual mouthparts because they were so wrinkled, folded, and tightly packed together (Pl. II, FIG. 12; Pl. IV, FIGS. 20B, 20D).

When the above specimens were placed in the K.A.A.D. mixture striking changes, apparently paralleling the process of metamorphosis, occurred, and the author had the opportunity of seeing the events apparently occurring in normal pupation much speeded up. The K. A.A.D. mixture penetrates the cuticle very rapidly causing the pupa and its parts to swell and inflate.

EXPLANATION OF PLATE VI

All of the figures were drawn with a binocular microscope equipped with a 9X ocular and a 3X objective with the aid of a camera lucida. Figures 1A through 6A and 1C through 6C correspond to Figures 1 through 6, Plate I.

FIGS. 1A., 1C. Ventral views of the head and thorax, and the abdomen respectively of a one day-old larva.

FIGS. 2A., 2C. Ventral views of the head and thorax, and the abdomen respectively of an early two day-old larva.

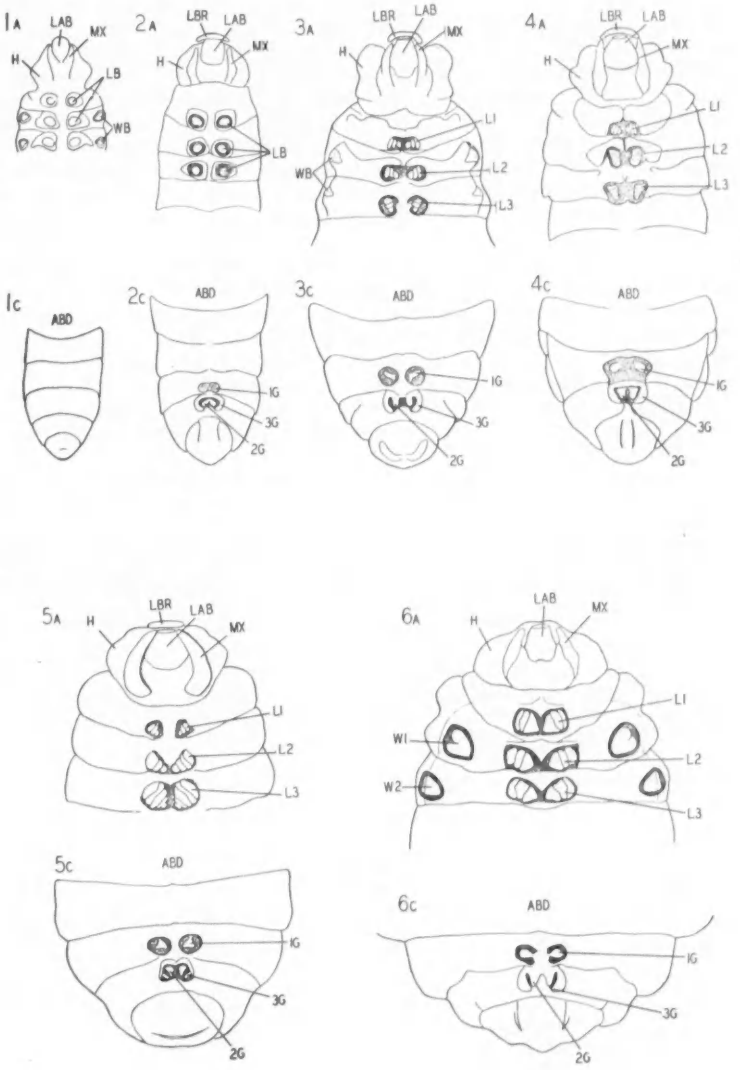
FIGS. 3A., 3C. Ventral views of the head and thorax, and the abdomen respectively of a late two day-old larva.

FIGS. 4A., 4C. Ventral views of the head and thorax, and the abdomen respectively of an early three day-old larva.

FIGS. 5A., 5C. Ventral views of the head and thorax, and the abdomen respectively of a medium three day-old larva.

FIGS. 6A., 6C. Ventral views of the head and thorax, and abdomen respectively of a late three day-old larva.

PLATE VI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The wings started to unfold and soon became free from the other thoracic parts. They slowly rotated caudad until the fore wings covered the hind wings. At the same time the head began to inflate and the mouthparts began to unfold and to rotate in an arc first ventrad, then caudad. The legs also began to expand under the influence of the killing solution and their distal segments became free of the thorax ventrally. As they became free they broadened, lengthened, and changed from their ventral position to a dorso-lateral one; the mesothoracic legs pushed up under the wings. The distal antennal segments which projected cephalad in the advanced prepupa elongated and rotated backwards until they extended caudad. This sequence of events is shown in a series of illustrations (Pl. II, FIGS. 11, 12, 13, 14, 15; Pl. III, FIGS. 16, 16A).

The adult form is nearly attained in the early pupa. The labium projects caudad from beneath the mandibles to the junction of the thorax and abdomen. The maxillae are now segmented into a proximal region (stipes) and a distal region (galea). The labial palpi are now divided into two, long proximal segments and two very short distal segments which usually extend at an angle laterad to the proximal segments. The mandibles cover the bases of the maxillae, labial palpi, and labium, and covering the base of the mandibles is the small flap of labrum.

The wings are now lying at a 45° ventro-caudad angle. Wing venation is not clear at this time because of the partial swelling of the wings.

The proximal segments of the antennae, the scapes, extend laterad to the level of the eye from the mid-line of the front of the head, while the other eleven segments extend directly caudad.

The segments of the legs project ventro-caudad at about the same angle as the wings.

The abdominal segments are beginning to taper caudally and the gonapophyses are folded together on the ventral surface of the terminal segments of the abdomen. All of these changes are shown in (Pl. III, FIGS. 17, 17A).

EXPLANATION OF PLATE VII

All of the figures were drawn with a binocular microscope equipped with a 12.5X ocular and a 1X objective with the aid of a camera lucida. Figures 7A through 10A and 7C through 10C correspond to Figures 7 through 10, Plate I.

FIGS. 7A., 7C. Ventral views of the head and thorax, and the abdomen respectively of a four day-old larva.

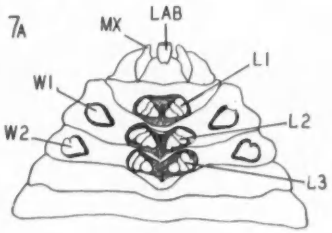
FIGS. 8A, 8C. Ventral views of the head and thorax, and the abdomen respectively of a five day-old larva or "day of capping" larva.

FIGS. 9A., 9C. Ventral views of the head and thorax, and the abdomen respectively of a six day-old larva or a "one day after capping" larva.

FIGS. 10A., 10C. Ventral views of the head and thorax, and the abdomen respectively of a seven day-old larva or a "two days after capping" larva.

All of the figures showing the internal structures in the honey bee (Plates VIII through XII) were drawn from sections with the aid of a micro-projector and all are magnified about 18 times.

PLATE VII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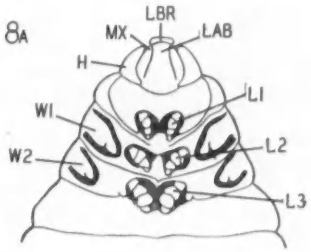
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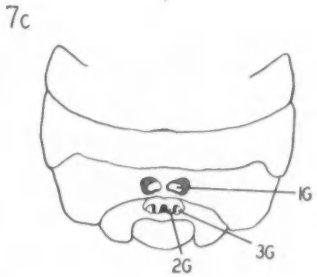
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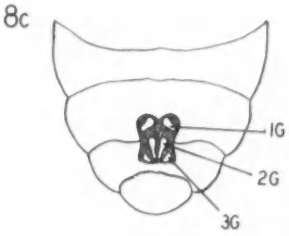
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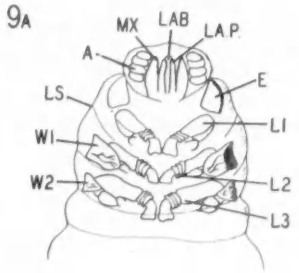
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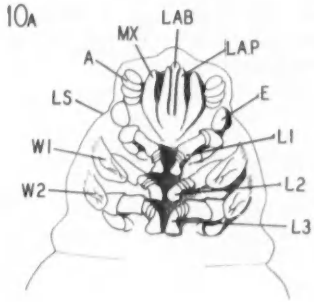
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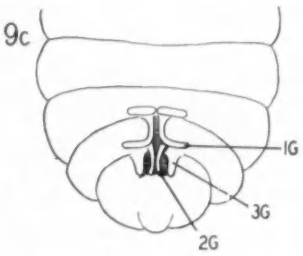
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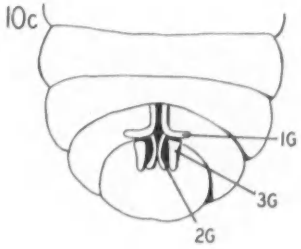
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Nine days after hatching (four days after capping), pupa. By this time the pupal cuticle has separated from the underlying imaginal cuticle and covers the body and all the appendages as a thin transparent layer. The imaginal appendages look more slender and segmentation of all the parts is more complete.

The gonapophyses have differentiated into the stinging organ. The first, second, and third pairs of gonapophyses becoming the lancets, sheath, and sting palpi respectively (Pl. III, FIGS. 18, 19, 18C, 18D).

As the pupa continues its development from this point the changes in form are rather minor, the most apparent being the change in the color of the body parts. The final (sixth) moult occurs about nine days after the fifth larval moult after which the imago emerges from its cell.

SUMMARY AND CONCLUSIONS

1. In the development of the larval honey bee there are certain characteristic "landmark" tissues that appear early in the post-embryonic development, such as wing buds and leg buds.

2. The wing buds and leg buds increase in size and change gradually in form during the process of metamorphosis.

3. The legs and wings develop in pockets in the hypodermis from about two days after hatching through five days after hatching. Leg segmentation also becomes apparent during this time.

4. During the first three days of capping the larval head and its appendages lengthen. The prothorax at first protrudes dorsally over the head, then recedes. The thorax decreases in diameter and the larval and pupal cuticles become separated and the space between them fills with moulting fluid.

5. After the shedding of the last larval skin the wrinkled sac-like appendages become inflated and the imaginal form is nearly attained.

EXPLANATION OF PLATE VIII

Figures 21 through 24 are of a full grown "day of capping" larva. Figures 25 and 26 are of a "one day after capping" larva.

FIG. 21. Longitudinal section through the head and thorax of a "day of capping" larva.

FIG. 22. Longitudinal section approximately 153 microns median to the section represented by Fig. 21.

FIG. 23. Longitudinal section about 288 microns median to the section represented by FIG. 21.

FIG. 24. Longitudinal section of a "day of capping" larva about 340 microns median to that represented by FIG. 21.

FIG. 25. Longitudinal section of a "one day after capping" larva.

FIG. 26. Longitudinal section about 460 microns median to the section represented by FIG. 25.

Figures 27, 28, 29 are drawings of a "two days after capping" larva.

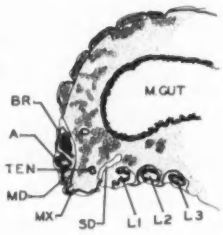
FIG. 27. Longitudinal section of the head and thorax showing the separation of the larval cuticle from the pupal cuticle.

FIG. 28. Longitudinal section approximately 208 microns median to the section represented by Fig. 27.

FIG. 29. Longitudinal section 88 microns median to the section represented by FIG. 28.

PLATE VIII

21



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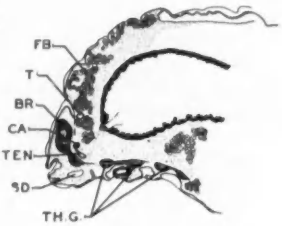

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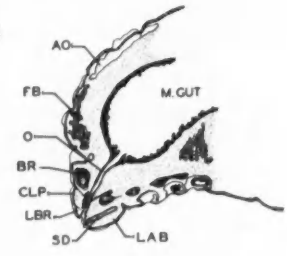

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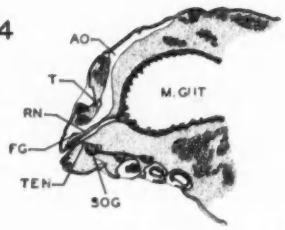

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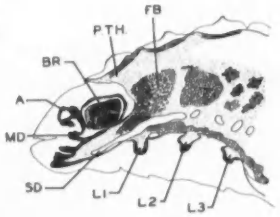

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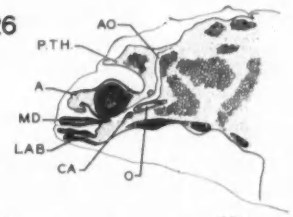

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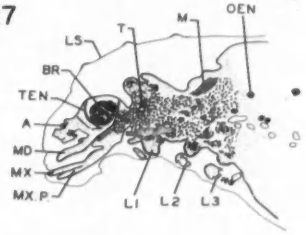

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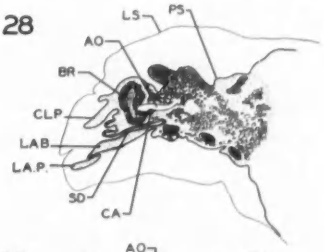

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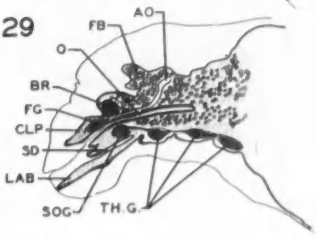

The head, mouthparts, and legs all rotate from an anterior to a ventro-caudal angle.

6. The late pupa, "four days after capping," appears morphologically like an adult. All of the appendages of the head, thorax, and abdomen are complete.

PART II

CUTICULAR AND INTERNAL STRUCTURAL CHANGES DURING LATE LARVAL AND PUPAL DEVELOPMENT

The author has illustrated the day to day internal changes in the head and thorax by a series of drawings of sectioned material. These drawings emphasize the cuticular modifications which correlate with the external changes previously described. They also show the changes in the relationship of the internal organs of the head and thorax from "day of capping" through "four days after capping". The histology of metamorphosis in the honey bee has been done by a number of investigators: Anglas (1901), McIndoo (1914), Koehler (1923), Snodgrass (1925), Oertel (1930), Powers and Melampy (1943), Lotmar (1945), and Dobrovsky (1950). Therefore, this paper does not deal primarily with histological aspects, but rather it is a study of the gross anatomical relationships of matamorphosis.

LARVAL STRUCTURES

The five day-old larva (day of capping). The head of this larva is conical in shape and is separated from the thorax by a constriction of the cuticle. The head is about as long as it is broad and only about

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EXPLANATION OF PLATE IX

Figures 30, 31, 32 are drawings of a "three days after capping" larva.

FIG. 30. This section shows the constriction between the head and thorax.

FIG. 31. This is a section about 340 microns median to the section represented by FIG. 30.

FIG. 32. This section is about 80 microns median to the section represented by FIG. 31.

Figures 33 through 37 are drawings of a "three days after capping" pupa. This animal is in the process of shedding the last larval skin. The head and thorax are undergoing rather dynamic changes in shape.

FIG. 33. This section shows the head at a ventro-caudal angle. The mouthparts and antennae also are extending in a ventro-caudal direction.

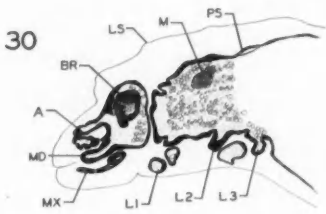
FIG. 34. This section is approximately 90 microns median to the section represented by FIG. 33. This shows the tentorium forming and the caudad extension of the maxillae. The leg rudiments also are extending caudally as opposed to the more ventral extension of the legs in the younger prepupae and larvae.

FIG. 35. This section is approximately 80 microns median to the section represented by FIG. 34. This drawing shows the caudad extension of the mouthparts, the change in shape of the cuticle of the thorax, and the narrow tubular midgut.

FIG. 36. This section is approximately 36 microns median to that represented by FIG. 35.

FIG. 37. This section is about 72 microns median to the section represented by FIG. 36.

PLATE IX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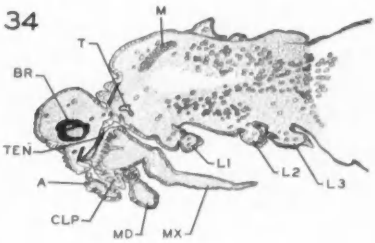
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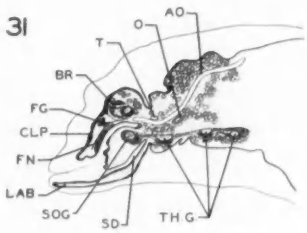
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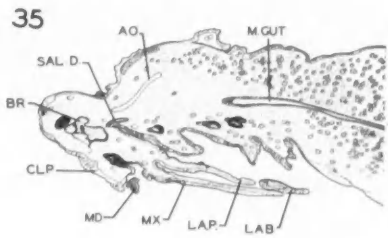
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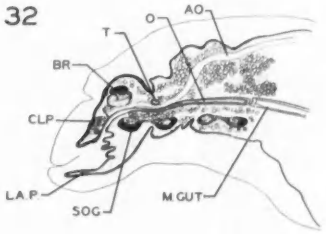
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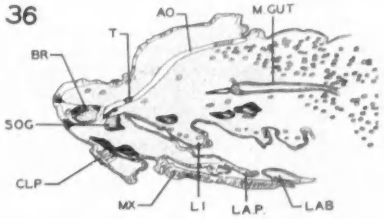
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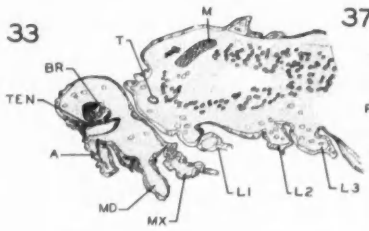
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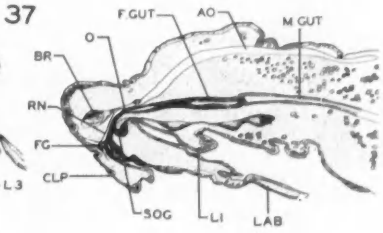
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one half as broad as the thorax. The imaginal antennae are developing in hypodermal pockets and even though they are short they are segmented (Pl. VIII, FIG. 21). The mandibles are conical structures located ventral to the antennae. The maxillae are developing ventral to the mandibles and extend cephalad. The clypeus and labrum are distinguished by cuticular constrictions (Pl. VIII, FIG. 23).

The legs and wings and their development in the hypodermal pockets of the thorax can be seen in (Pl. VIII, FIG. 21; X, FIGS. 42, 43). The larval cuticle is separating from the pupal cuticle in the regions of the head and thorax.

Internal structures. In the five day-old larva the fat bodies are scattered in groups throughout the thorax, but very few are in the head proper.

The tentorium may be seen as a tubular structure at the base of the head posterior to the suboesophageal ganglion (Pl. VIII, FIGS. 21, 22). The corpora allata are spherical bodies lying posterior to the brain and above the tentorium (Pl. VIII, FIG. 22).

The dorsal vessel (heart) is located on the dorsal mid-line just beneath the cuticle in the abdomen and thorax. It follows the cuticle to the level of the prothorax where it turns ventrad to the level of the oesophagus. It then turns cephalad and runs beneath the brain (Pl. VIII, FIGS. 23, 24).

EXPLANATION OF PLATE X

FIG. 38. This is a drawing of a section of an early pupa, showing the rather unusual folding of the epithelium of the midgut near its ventral junction with the foregut. Note the more characteristic adult appearance of the thoracic region at this stage of development. The fat bodies are numerous and equally distributed among the internal organs.

Figures 39, 40, 41 are drawings of sections of a "four days after capping" pupa.

FIG. 39. Longitudinal section of the head and thorax showing the nearly adult shape.

FIG. 40. This is a section approximately 216 microns median to that represented by FIG. 39. Note the fat body distribution, the shape of the mouthparts, the shape of the midgut, and the position of the aorta in the head and thorax.

FIG. 41. This is a section about 216 microns median to the section represented by FIG. 39. This drawing shows the thoracic and abdominal regions. Note the constriction which separates them. The heart in the abdomen and thorax is shown following the contour of the constricted cuticle. Note the depth of the aorta in the thorax and the superficial position of the heart in the abdomen. Oenocytes are concentrated in the abdominal region.

Figures 42 and 43 are cross section drawings of a "day of capping" larva.

Figures 44 and 45 are cross section drawings of a "one day after capping" larva.

FIG. 42. Cross section through the thorax which shows the wings and legs growing in the cuticular pockets.

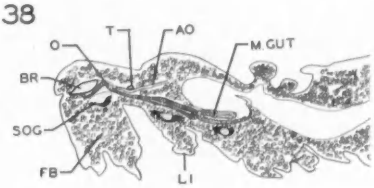
FIG. 43. Cross section about 350 microns anterior to that represented by FIG. 42.

FIG. 44. Cross section through the thorax of a "one day after capping" larva showing the attachment of the wings to the thorax. Note the constricted oesophagus and the dorsal position of the aorta.

FIG. 45. Cross section through the thorax about 258 microns anterior to the section represented by FIG. 44. This drawing shows the head covered by a prothoracic shield. The silk ducts are greater in diameter than the oesophagus at this level.

PLATE X

38



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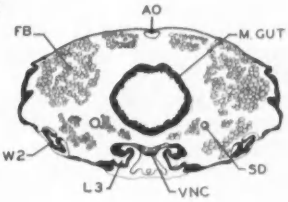

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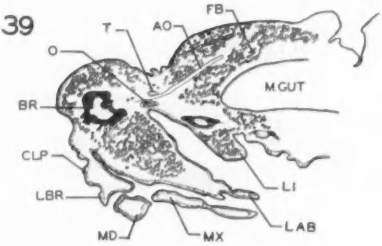

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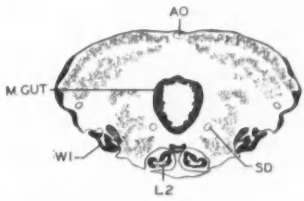

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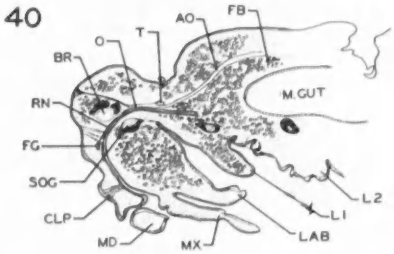

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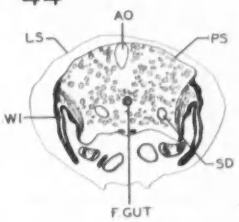

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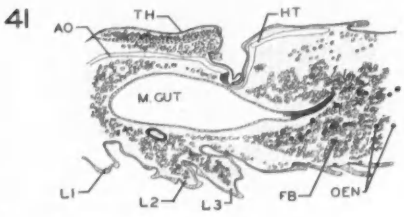

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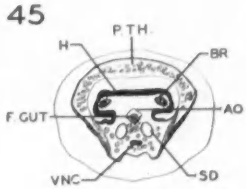

The silk glands and their ducts run anterior from the thorax to the head, beneath the tentorium and suboesophageal ganglion, and to an opening at the tip of the labium-hypopharynx. The labium-hypopharynx complex is described in detail by Dobrovsky (1950).

The brain, suboesophageal ganglion, and the thoracic ganglia may be seen in their respective positions in the larva (Pl. VIII, FIGS. 22, 23, 24).

The oesophagus is a narrow tubular structure running in a caudal direction where it joins the midgut in the prothorax. The midgut, as described by Dobrovsky (1950), is a large "crescent shaped tubular bag" about one-third to one-half the diameter of the body (Pl. VIII, FIGS. 21, 22, 23).

The six and seven day-old larvae. The changes that occur during the sixth and seventh days of larval life may be seen in (Pl. VIII, FIGS. 25, 26, 27, 28, 29; Pl. X, FIGS. 44, 45; Pl. XI, FIGS. 46, 47, 48).

The eight day-old larva (three days after capping), late prepupa. The constriction between the head and thorax has deepened so that the connection between the two regions is now smaller in diameter than the width of the head. The lengthening of the antennae and the curves in them are shown in (Pl. IX, FIG. 30). The head is still lengthening dorso-ventrally and also cephalo-caudally. The clypeus is now a prominent ventro-cephalic extension of the front of the head into a long upper lip-like projection. The mandibles, maxillae, labial palpi, and labium are slightly longer and more slender than in the previous stage (Pl. IX, FIGS. 30, 31, 32).

The tibia and tarsus of the mesothoracic leg can be seen in cross section in (Pl. XI, FIG. 49). The attachments of the legs and the wings to the thorax are shown in cross section (Pl. XI, FIGS. 50, 51).

Internal changes. The fat bodies are now found scattered in the head but they are not in any of the head appendages.

The dorsal vessel does not follow the dorsal cuticle of the thorax as closely as it did in the "day of capping" larva. It is now imbedded

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EXPLANATION OF PLATE XI

Figures 46, 47, and 48 are drawings of a "one day after capping" larva. Figures 49, 50, and 51 are drawings of a "three days after capping" larva.

FIG. 46. Cross section drawing 276 microns anterior to the section represented by FIG. 45. This shows the corpora allata and their relationship to the aorta.

FIG. 47. Cross section 78 microns anterior to that shown in the previous figure.

FIG. 48. Cross section through the head about 54 microns anterior to that shown in FIG. 47. This drawing shows the proportionate size of the head and brain. The fat bodies are very scarce at this level.

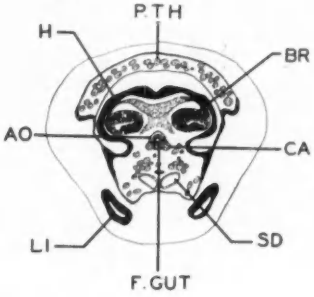
FIG. 49. Cross section through the thorax of a "three days after capping" larva. Note the definite segmentation of the legs, the loose outer larval cuticle, and the abundance of fat bodies.

FIG. 50. Cross section about 900 microns anterior to the section represented by FIG. 49. The wing and leg body attachments are shown. The aorta at this level is about midway between the dorsal body wall and the oesophagus. The silk ducts are shown lying laterally to the oesophagus.

FIG. 51. Cross section about 130 microns anterior to the section represented by FIG. 50. Note the closeness of the aorta, oesophagus, and ventral nerve cord.

PLATE XI

46



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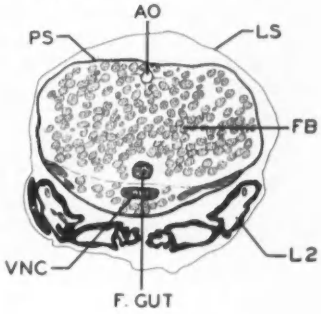

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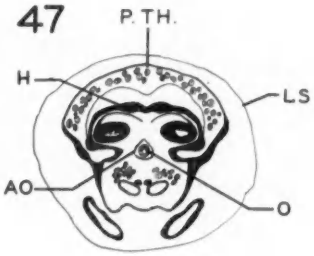

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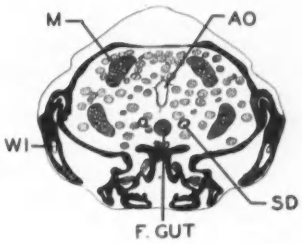

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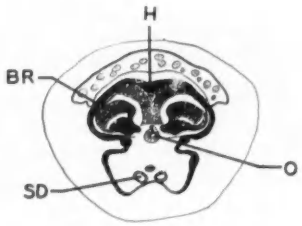

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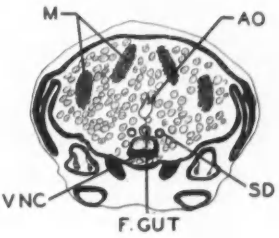

in the fat bodies a short distance ventral to the cuticle. As the aorta reaches the metathorax it runs at a ventro-cephalic angle to the level of the oesophagus, then turns cephalad beneath the brain (Pl. IX, FIGS. 31, 32; Pl. XI FIGS. 50, 51).

The mouth opening is between the clypeus-labrum and labium. The pharynx is a narrow, tubular opening running dorsally a short distance, then caudally where it meets the oesophagus. The oesophagus extends caudad into the abdomen where it abuts with the midgut. The oesophagus and midgut are both still narrow tubes (Pl. IX, FIG. 32; Pl. XI, FIGS. 49, 50, 51).

Muscle tissue is present in the thorax in larger bundles (Pl. IX, FIG. 30; Pl. XI, FIGS. 50, 51).

The silk ducts and their relationship to the other mouthparts may be seen in cross section. The silk ducts lie lateral to the oesophagus in the thorax (Pl. XI, FIGS. 50, 51) and unite, forming a single duct at the caudal extremity of the labrum (Pl. XII, FIGS. 53, 54).

A cross section at the level of the prothoracic spiracles shows the tracheal tubes extending between them. Directly ventral to the trachea in the mid-line the aorta can be seen. It is flattened between the trachea and the oesophagus (Pl. XII, FIG. 52).

The tentorial arms, and their relationship as supportive structures, may be seen in cross section as tubes lying lateral to the silk duct and united by a connecting arm that arches above the silk duct (Pl. XII, Fig. 53). The lateral tentorial arms run cephalad from the caudal extremity of the head, lateral to the suboesophageal ganglion, and unite with the cuticle in the region of the base of the antennae.

The maxillae and labium originate at about the same level of the ventro-caudal base of the head. The labium is a broad, ventral ex-

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EXPLANATION OF PLATE XII

Figures 52 through 57 are drawings of a "three days after capping" larva.

FIG. 52. This section is at the level of the prothoracic spiracles and the transverse tracheal connective. It is about 340 microns anterior to that represented by FIG. 51.

FIG. 53. Cross section about 160 microns anterior to the section represented by FIG. 52. The transverse arm of the tentorium is shown as a solid rod-like arch connecting two hollow discs that lie on either side of the silk duct. These hollow discs are the longitudinal tentorial arms also shown in FIG. 34 in longitudinal section.

FIG. 54. Cross section approximately 110 microns anterior to the section represented by FIG. 53. The eyes appear as discs attached to the lateral surfaces of the head. The optic lobes of the brain are shown as well as the suboesophageal ganglion. Note the protruding maxillae.

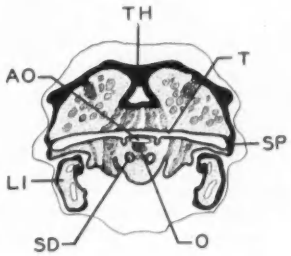
FIG. 55. This section is 60 microns anterior to that represented by FIG. 54. The eyes and optic lobes are very prominent. Note also the suboesophageal ganglion, oesophagus, maxillae, labium, and labial palpi.

FIG. 56. This section is approximately 100 microns anterior to the section represented by FIG. 55. The optic lobes, brain, and the oesophagus are shown. Note also the mouthparts.

FIG. 57. This section is approximately 350 microns anterior to that represented by FIG. 56. This section shows the mouthparts very clearly and their relationship to each other just previous to pupation. The antennae, mandibles, maxillae, labial palpi, and labium are shown.

PLATE XII

52



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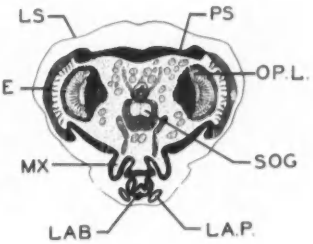

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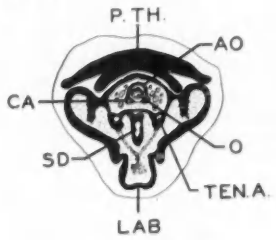

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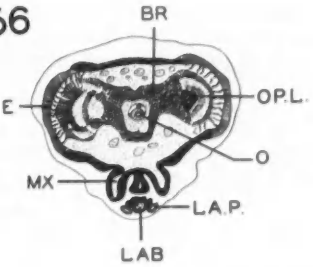

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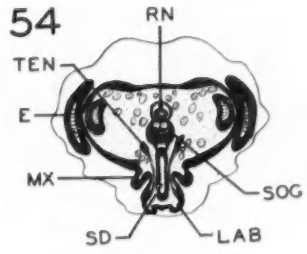

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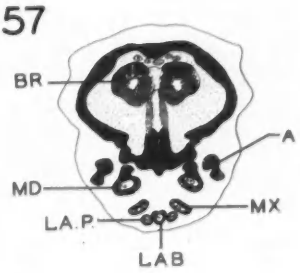

TABLE SHOWING THE MOULTS OF THE WORKER HONEY BEE AND THE MORPHOLOGICAL DEVELOPMENT CHARACTERISTIC OF THE INSTAR

DAY*	STAGES*	MOULT*	HEAD	THORAX	ABDOMEN
1 2 3	Egg				
4	1st Larval	1st Moult	Larval mouthparts distinguishable in the head. Pl. V, Fig. 1B.	Imaginal wings and legs visible as spots of tissue beneath the larval cuticle. Pl. VI, Figs. 1A, 1C.	
5	2nd Larval	2nd Moult	Head Larger. Pl. V, Figs. 2B, 3B.	Legs beginning to show segmentation. Pl. VI, Figs. 2A, 3A.	Gonapophyses visible on midventral side of the posterior abdominal segments. Pl. VI, Figs. 2C, 3C.
6	3rd Larval	3rd Moult	Head size unchanged.	Legs and wings developing in hypodermal pockets. Pl. VI, Figs. 4A, 5A, 6A.	Gonapophyses developing in hypodermal pockets. Pl. VI, Figs. 4C, 5C, 6C.
7	4th Larval	4th Moult	Head size unchanged Pl. V, Fig. 7B.	Enlarging. Pl. VII, Fig. 7A.	Enlarging. Pl. VII, Fig. 7C.
8	5th Larval Day of capping		Head size unchanged Pl. V, Fig. 8B.	Enlarging. Pl. VII, Fig. 8A.	Enlarging. Pl. VII, Fig. 8C.
9	5th Larval 1 day after capping (Prepupa)		Mouthparts elongating cephalad. Imaginal eye visible on side of head. Plate V; Fig. 9 B.	Legs longer and individual segments recognizeable. Wing venation now apparent. Plate VII Fig. 9A.	Gonapophyses longer-3 pairs differentiating Pl. VII, Fig. 9C.
10	5th Larval 2 days after capping (Prepupa)		Larval cuticle separating from pupal cuticle. Pl. V, Fig. 10B.	Larval cuticle separating from pupal cuticle. Pl. VII, Fig. 10A.	Gonapophyses longer and more slender. Pl. VII, Fig. 10C.
11	5th Larval 3 days after capping (Late Prepupa)	5th Moult	Head broadening. Eyes larger and wrinkled. Pl. IV, Fig. 11B.	Larval cuticle completely loose from pupal cuticle. Wings long and wrinkled. Pl. IV, Figs. 11B, 11A.	Larval cuticle loose from pupal cuticle. Abdomen tapering caudally. Pl. IV, Figs. 11C, 11D.
11	Early Pupal 3 days afterc apping		Head enlarging. Mouthparts rotating ventrally and caudally. Pl. II, Figs. 12, 13, 14, 15.	Legs elongating and extending ventro-caudad.	Abdomen tapering caudally. Gonapophyses tightly folded. Pl. II, Figs. 12, 13, 14, 15.
12	Pupal 4 days after capping		Head adult-like. Mouthparts segmented. Pl. III, Fig. 18.	Imaginal legs slender and clearly segmented. Appendages encased in thin pupal cuticle. Pl. III, Fig. 19.	Stinging organ now formed from gonopophyses. Pl. III, Figs. 18C, 18D.
20	Pupal	6th Moult			
21	Adult				

*Data after Bertholf (1925).

tension of the head, flanked by two short cuticular outpocketings, the maxillae (Pl. XII, FIGS. 53, 54, 55).

An anterior section shows the labium free from the head and bounded laterally by the tubular labial palpi. The maxillae are still joined to the head proper but are longer and lie dorso-lateral to the labium and labial palpi (Pl. XII, FIGS. 55, 56).

Anterior to the junction of the antennae and mandibles with the head, a cross section shows the tubular labium in the mid-ventral line flanked by the labial palpi, the maxillae dorso-lateral to the labial palpi, the mandibles dorso-lateral to the maxillae, and the antennae lateral to the mandibles (Pl. XII, FIG. 57).

The eyes, as seen in cross section, are saucer-shaped discs on the sides of the head (Pl. XII, FIG. 54). Anteriorly the optic lobes of the brain and the nerve connections with the cuticle on the compound eyes become apparent (Pl. XII, FIGS. 55, 56). The optic lobe nerve complex has been described and illustrated by Ionescu (1902).

PUPATION

The early pupa, just after the completion of the last larval moult, has a thickened, wrinkled cuticle. According to Power and Melampy (1943) the surface of "day of pupation" bees is impenetrable to stains. The writer also found that the cuticle in sectioned "day of pupation" bees did not absorb stains. The cells of the hypodermis are long and narrow and appear to be in a state of secreting which might account for this inability to absorb.

The head is now much longer dorso-ventrally and has enlarged to nearly the adult size and shape. The enlarged head emphasizes the constriction between it and the narrowed prothorax. However, there is still no apparent constriction between the thoracic and abdominal segments (Pl. IX, FIGS. 34, 35). The legs, whose proximal segments in the larva and prepupa extended ventrad, now extend ventro-caudad.

The labium, labial palpi, and maxillae are now projecting caudad (Pl. IX, FIGS. 34, 35, 36, 37). The proximal segments of the antennae (scapes) and the mandibles extend ventro-caudally (Pl. IX, FIGS. 33, 34), The clypeus-labrum flap which is located between the antennae on the front of the head extends more sharply caudad than the mandibles (Pl. IX, FIGS. 36, 37).

The adult tentorium can be seen forming by the invagination of the cuticular hypodermis of the head. The anterior pits form immediately ventral to the base of the antennae, and the posterior pits form at the base of the maxillary attachments to the head (Pl. IX, FIG. 34). This seems to be the only account of the formation of the tentorium during early pupation. The tentorial arms of the adult bee are illustrated by Snodgrass (1925).

Internal changes. The enlargement of the head makes the brain appear proportionately smaller than in the prepupa (Pl. IX, FIGS. 33, 34, 35). The frontal ganglion and the recurrent nerve can be seen in the head at the base of the clypeus (Pl. IX, FIG. 37).

The thoracic muscle bundles are becoming elongate and are now in contact with the prothoracic cuticle (Pl. IX, FIGS 33, 34, 35).

The dorsal vessel, in the abdomen, lies close to the cuticle, but in the thorax it extends at a ventro-cephalic angle until it reaches the oesophagus, then it passes beneath the transverse prothoracic trachea and runs cephalad into the head. Some of the circular muscles of the aorta wall can be seen in the metathorax (Pl. IX, FIGS. 36, 37).

The midgut is still very narrow and it is lined with a thin layer of epithelium. It joins the oesophagus in the metathoracic segment (Pl. IX, FIG. 37).

A few fat bodies are scattered throughout the head and thorax, but they are more concentrated in the abdomen (Pl. IX, FIG. 35).

Late three days after capping, pupa. The animal at this stage has made some further advancements in form. The folds and constrictions at the junction of the thorax and abdomen reveal a more mature shape. The ventral cuticle of the abdomen has formed overlapping sternal plates (Pl. IX, FIG. 38).

The major internal changes at this stage are the even distribution of many fat bodies throughout the head, thorax, and abdomen and the nature of the midgut. The entrance to the midgut is on the ventral surface of the honey stomach, through a narrow, tubular midgut extension, lying ventral to, and separated from, the midgut proper by a fold in the epithelium (Pl. IX, FIG. 36).

Four days after capping, pupa. By this time most of the adult characteristics are determined. The thorax and abdomen are separated by a deep dorsal fold. The sternal plates of the abdomen are more completely formed and are beginning to overlap (Pl. X, FIG. 41).

The fat bodies are numerous, small, and distributed equally in the head, thorax, and abdomen. The honey stomach midgut extends well into the thoracic region and is now an enlarged sac-like structure. The epithelium of the midgut is more definitely formed than in the previous pupal stage.

The heart follows the cuticle through the constriction of the thorax and abdomen and then the aorta proceeds anteriorly and imbeds in the thorax about half way between the dorsal cuticle and the midgut. In the prothorax the aorta runs at a gentle ventral angle until it passes beneath the prothoracic trachea and then into the head (Pl. X, FIGS. 39, 40, 41).

SUMMARY AND CONCLUSIONS

1. The larval midgut is about one-half the diameter of the thorax. The head, as seen in section, is short in length as are its appendages. The hypodermal pockets containing the legs and wings can also be seen in section.

2. The silk glands and ducts originate in the abdomen, pass through the thorax laterally to the oesophagus, and unite in the labium as a single canal.

3. The brain occupies more space proportionately in the head during the late larval stages.

4. The larval heart and aorta run from posterior to anterior along the dorsal mid-line of the abdomen and thorax respectively. In the pupa the aorta is imbedded in the fat bodies about half the distance between the dorsal cuticle and the oesophagus.

5. The pupal tentorium arises as tubular invaginations of the anterior and posterior surfaces of the head.

6. The oesophagus elongates during the prepupal stage until it extends well into the thorax.

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Snodgrass, R. E. 1925. Anatomy and physiology of the honeybee. New York: McGraw-Hill Book Co., Inc., 327 pp.

THE MICROTOMIST'S FORMULARY AND GUIDE. PETER GRAY. 1954. The Blakiston Co., Inc., New York. xiii + 794. Price, $10.50.

This work is an enormous compilation, taken in general from the entire field of microtechnique and certain related areas, but without the inclusion of a section on microscopy. It is the author's evident intention to leave microscopy to special treatises on the subject. The book is unusual in that it is meant to serve both the beginner, for whom it gives elementary instructions, and the advanced worker, for whom it may serve as a source reference.

Chapters 1 to 16 comprise Part 1 and introduce the beginner to the making of dry, fluid, an solid wholemounts; smear and squash preparations; ground, free, frozen, paraffin, and nitrocellulose sections; double embedding; injection methods; and staining. Finishing steps for each technique and the use of essential items of equipment are included.

Chapters 17 to 28 comprise Part 2 and deal with preservatives, fixatives, fixative accessories, general and special dye stains, dye stain accessories, metal stains, metal stain accessories, solvents and oils, mounting media, embedding media, and miscellaneous formulas.

The rather elaborate, coded reference system may be disconcerting to some readers, but it is fully explained and will no doubt be very useful to those making extensive use of the book. Especially valuable are the tabular summaries on defects appearing during sectioning, section mounting, and after staining and mounting the cover glass; on the preparation of fixatives from basal solutions; and on the physical properties of dehydrating agents, essential oils, synthetic clearing agents, and universal solvents.

Entomologists will be especially interested in methods for insects, which include neurological preparations (silver impregnations and complex stains),

differential muscle staining in larvae, softening and differential staining of chitin, fixation, tracheal injection, skeletonizing, and the preparation of wholemounts.

Although this book contains a vast array of material and gives detailed instructions for many procedures, some omissions and opinions may strike the reader rather glaringly, even after the author's graceful acknowledgement of such possibilities. It will seem odd that the author objects to the use of Bouin's fixative (except for nuclei), objects to dioxane as a dehydrating agent, and prefers Canada balsam to clarite and piccolyte as mounting media. The procedure of melting the paraffin of mounted paraffin sections before passing them to xylene seems hardly necessary. Though the author cannot mean it, on page 273 his instructions call for transferring tissue from 70% alcohol to paraffin for embedding. While the intent is obvious to any technician, beginners may be understandably confused. Likely to be confusing also are the instructions of pages 128 to 132 where, although the topic-heading indicates staining with hematoxylin and eosin, the text describes the use of coelestin blue B and phloxine. Evidently the author regards as unnecessary the removal of the mordant from hematoxylin stained sections, for in contrast to common usage he does not stress a long wash at this point. Butyl alcohol methods for softening chitin are not mentioned. Neither have I been able to find any reference to the drop method of dehydration or to Flemming's triple stain. The entire subject of chromosome technique receives only scanty treatment. The author perhaps has his own reasons for much of this, the inclusion of which, it seems, would have been helpful to users of the book.

Lists of abbreviations, and of books and journals cited, are given. The index is both large and detailed. These, together with the special items already mentioned, should make the book very useful in spite of its errors and omissions. In fact, because of its enormous scope, it seems to the reviewer that for workers in some fields it may well be indispensable. Biologists owe an immense debt of thanks to the author for the many long hours of library work that he has saved them.—RAY MOREE

AUTOTROPHIC MICRO-ORGANISMS by B. A. FRY and J. L. PEEL (Editors) Pp. xi+305. Cambridge University Press, New York. April, 1954. Price $5.00.

This publication, the fourth in the series of international symposia sponsored and published by the Society for General Microbiology, consists of thirteen authoritative articles dealing with the classification, physiology, biochemistry, and economic importance of autotrophic micro-organisms. Eight of the articles are primarily concerned with autotrophic bacteria, two with nutrition and metabolism of the blue-green and green algae, two with aspects of photosynthesis, and one on mass cultures of algae.

Although the book is rather specialized and thus of greatest aid to those working in the field it has a number of features interesting to other scientists. It is instructive for any systematist to note the current problems in classification of the somewhat arbitrarily—defined autotrophic bacteria as presented by K. A. Bisset and Joyce B. Grace. In general these authors reemphasize the importance of morphologic characters while giving the reasons for the non-existence of genera of "peculiar" autotrophic bacteria; the invalid genera are believed to represent minor variants of single species, errors due to descriptions based on contaminated culture, or to consist of a valid algal genus wrongly placed in the bacteria. The difficulties in classification of these relatively simple organisms is well-presented. It is instructive—and fortunate—that in evolution the complexities of the organism did not increase in proportion to cell number.

Despite the title of the book there is no clear-cut and generally accepted definition of autotrophic micro-organism. In fact some forms, *eg. Chromatium* (Thiorhodoceae), may live autotrophically, requiring only inorganic substance when sulfur coumpounds are present, or may live heterotrophically if supplied with any of several organic acids. This situation is aptly expressed by D. D. Woods and June Lascelles, authors of the introductory chapter, who say autotrophism is a way of life rather than a basis for grouping organisms.

This point is emphasized by the absence of information in the book on nutritional types of the algae commensurate with those of the bacteria. In the

latter there are phototrophic and chemotrophic forms, each being further classified as requiring or not requiring organic substances. However, how commonly these nutritional types occur in the algae is not presently known. In view of the wide range of nutritional types in the bacteria—as well as the range of behavior within a species—it would not be unexpected that a similar range may exist in the algae. For example many pigmented algae may be phototrophic but require organic substances; also pigmented or colorless chemotrophs may be found. To some extent this expectation may be borne out by corrections in present classificatior, as pointed out by G. E. Fogg and Miriam Wolfe in their review of nitrogen nutrition of the blue-green algae, since certain known chemotrophs such as *Beggiatoa* and *Vitreoscilla* may be allied to the blue-green algae rather than to the bacteria.

One of the unifying aspects of the several articles is the similarity of metabolic steps in autotrophic and heterotrophic forms. These similarities are revealed in Han's Guffron's excellent review of photosynthesis, and in the articles on photosynthetic activity of bacteria by H. Larsen and S. R. Elsden.

On the whole the articles are well written and the book is relatively free of typographical errors. The selection of topics seems very good and a reasonably complete picture of current knowledge of the autotrophic way of life is presented.

NOE HIGINBOTHAM

MAILING DATES FOR THE 1954 ISSUE OF THE ANNALS

March Number—May 4, 1954.

June Number—August 17, 1954.

September Number—November 23, 1954.

December Number—February 18, 1955.

INDEX TO VOLUME 47

All new names are printed in *italics*

Acarina, 425, 646.
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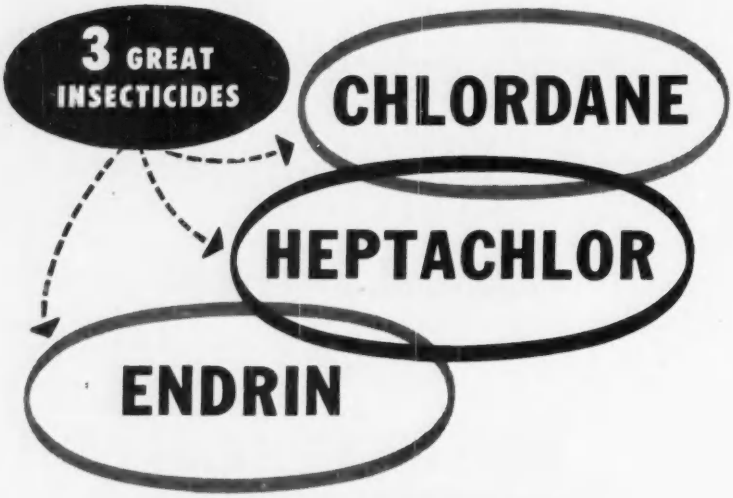

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